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Fungal Parasites of the Phytoplankton. II

(Studies on British Chytrids, XII)

BY

HILDA M. CANTER (MRS. J. W. G. LUND)

(*Freshwater Biological Laboratory, Ambleside, Westmorland*)

With Plates VIII–XI and fourteen Figures in the Text

ABSTRACT

This paper brings together the species of Chytrid fungi recorded from large bodies of water in the British Isles. Four new species are described.

THE presence of numerous fungi living as parasites and saprophytes on planktonic algae was noted in an earlier paper (Canter, 1950). The following species of chytrids have already been recorded from large bodies of water in the British Isles: *Dangeardia mammillata* (Ingold, 1940; Canter, 1946), *Amphicypellus elegans* (Ingold, 1944), *Endocoenobium eudorinae* (Ingold, 1940), *Chytriomyces tabellariae* (Canter, 1949), *Rhizophidium fragilariae*, *R. ephippium*, *R. sphaerocystidis*, *Rhizidium Windermerense*, *Chytridium versatile*, *Zygorhizidium melosirae* and *Z. parvum* (Canter, 1950). *Rhizophidium planktonicum* (Canter and Lund, 1948) is now considered by the writer to be an aggregate species. In the present paper further details of the occurrence and distribution of some of the fungi mentioned above are given and four other chytrids are described.

1. *RHIZOSIPHON CRASSUM* SCHERFFEL

Although *Anabaena* spp. are widely distributed and often occur in quantity in some lakes of the English Lake District (i.e. Esthwaite Water and Windermere, South Basin), this fungus has as yet been found in the Lake District only on *Anabaena* sp. (unidentified due to lack of akinetes), collected in Loweswater from June 1 to 13, 1949. The fungus seems to be widespread and not confined to one species of *Anabaena*. I have found it parasitizing *A. spiroides* Kleb. var. *crassa* Lemm. in Swithland Reservoir, Leicestershire, August 24, 1939 (sample preserved in 70 per cent. alcohol). B. M. Griffiths (1925) records a chytrid attacking the vegetative cells of *A. affanis* var. *intermedia* Griffiths in Norton Mere, Albrighton, August 1922. I have looked at this collection preserved in 2 per cent. formaldehyde and can identify the fungus as *Rhizosiphon crassum*. Again it was present in a preserved sample sent to me by Dr. W. Rodhe from Säbysjön, Sweden, collected July 16, 1944 (Text-fig. 4). Here it occurred on *Anabaena* spp. together with *Phlyctidium anabaenae* Rodhe et Skuja which is discussed later in this paper.

[Annals of Botany, N.S. Vol. XV, No. 58, April 1951.]

Owing to the dense pigmentation and the gas vacuoles in *Anabaena* it is often difficult in living material to observe the portions of the fungus which occur within the host cell, especially in the early stages of infection. However, staining with acetocarmine was found to be a satisfactory method for demonstrating the endobiotic system. The material (either living or previously fixed) is placed on a slide under a coverslip with very little water so that the algal filaments are slightly flattened. Acetocarmine stain is then run in and the slide heated for a few minutes until it is quite hot. By this technique the gas vacuoles disappear, the cell contents become colourless, while the fungus stains a deep purple.

Life-history[1]

The zoospore usually penetrates just into the mucilage sheath surrounding the *Anabaena* filament and then produces a fine thread to a vegetative host cell (Pl. I, Fig. A). Sometimes encystment of the zoospore on the outer surface of the mucilage sheath was observed. At first the encysted zoospore contains granular protoplasm and a small refractive globule. As the content passes into the algal cell the zoospore body and thread gradually empty (Text-fig. 1, A, B). The content passes in as a walled swelling directly continuous with the zoospore thread. This is well shown after staining (Text-fig. 1, C, D). As noted earlier, the young stages in the internal development of the fungus are extremely difficult to see in living material. However, by the time the fungus has grown a little, infected cells can be distinguished under low power since their content appears highly refractive, thus contrasting with the dull bluish-black hue of healthy cells. Text-fig. 1, C–I, drawn from stained material, show that the primary spherical internal body (prosporangium) enlarges at the expense of the cell in which it is embedded and that the rhizoidal system develops later. The latter consists of two wide undulate tubes (Text-fig. 1, I, and Pl. I, Fig. B), one emerging from either side of the prosporangium and extending through several host cells, so that up to eleven algal cells may be killed by one thallus. The rhizoids are more like lateral extensions of the prosporangium than are the majority of chytrid rhizoids which tend to be fine and tapering. The rhizoid up to 4μ broad may reach a length of 60μ (this measurement is the sum of the lengths of the two rhizoidal axes excluding the prosporangium). Usually the prosporangium (5–8μ in diameter) enlarges, until eventually it occupies the entire volume of the host cell. Occasionally slight hypertrophy of the host cells containing prosporangia was noticed. In a few prosporangia a large densely staining body (Text-fig. 1, J, K) was seen which may be the nucleus.

A sporangium is formed by gradual evacuation of the content from the prosporangium and rhizoidal system. It begins as a spherical hyaline bud (Text-fig. 1, L–N), which emerges just beside the place of penetration of the original zoospore thread. As the bud enlarges its contents cease to be hyaline

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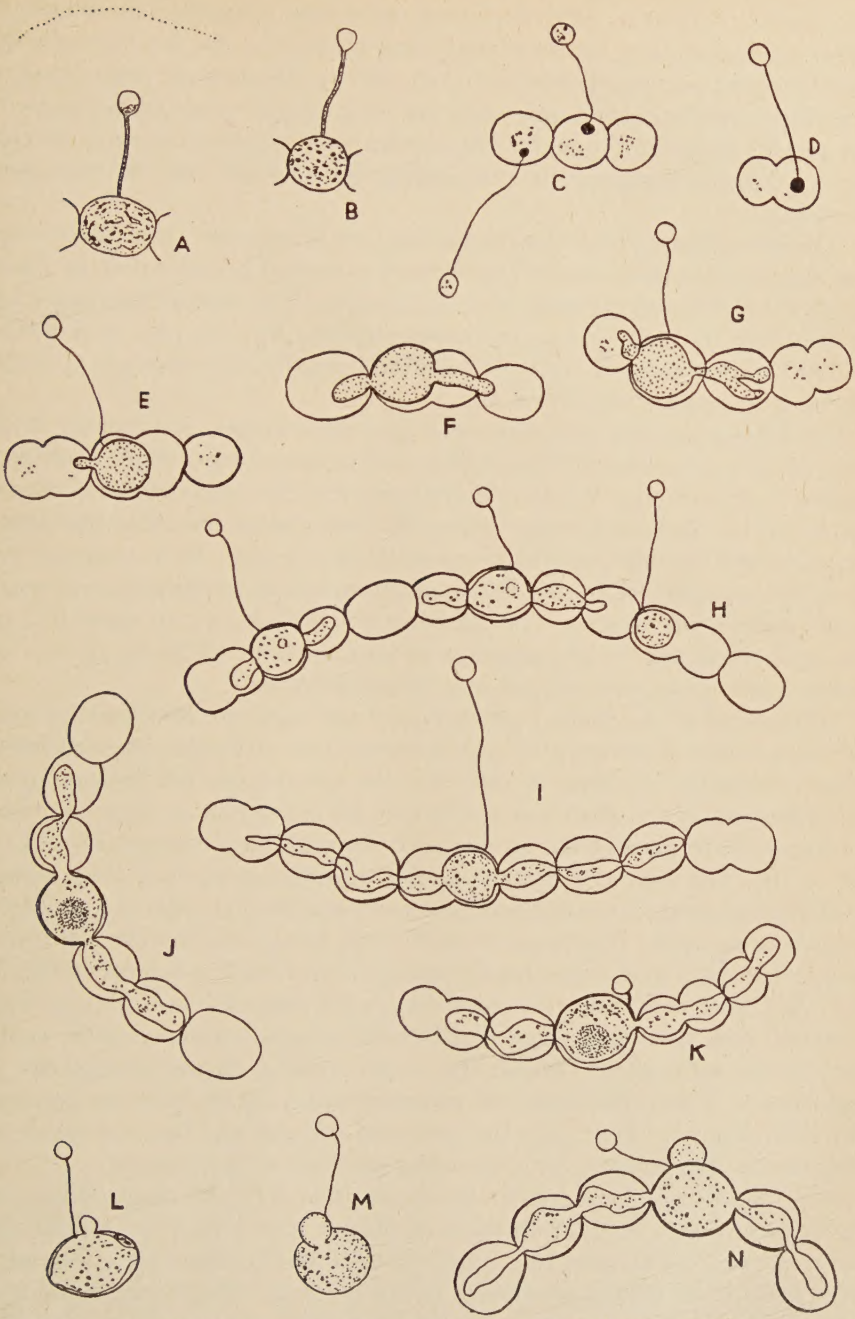
[1] The following description is based on material from Loweswater, no significant differences are exhibited by the fungus from other localities.



TEXT-FIG. 1, A–N. *Rhizosiphon crassum* Scherffel. A, B, recently encysted zoospores with a germ-tube; dotted line in (A) is edge of algal mucilage envelope. C, D, earliest stages in the development of the prosporangium. E–I, growth in size of the prosporangium and the development of the lateral rhizoidal system. J, K, prosporangia containing large nuclear body? L–N, first appearance of sporangium as a hyaline bud. C–K stained with acetocarmine. (All × 1100.)

but become granular with numerous refractive globules. At length the spherical sporangium becomes more flask-shaped by the development of a tubular apical portion (Text-fig. 2, C–G), and by this time the prosporangium and rhizoidal system have apparently lost most of their protoplasmic contents. As noted by Scherffel (1926) for *Rhizosiphon crassum*, in the course of development of the sporangium its protoplasm may contain one or more large vacuoles.

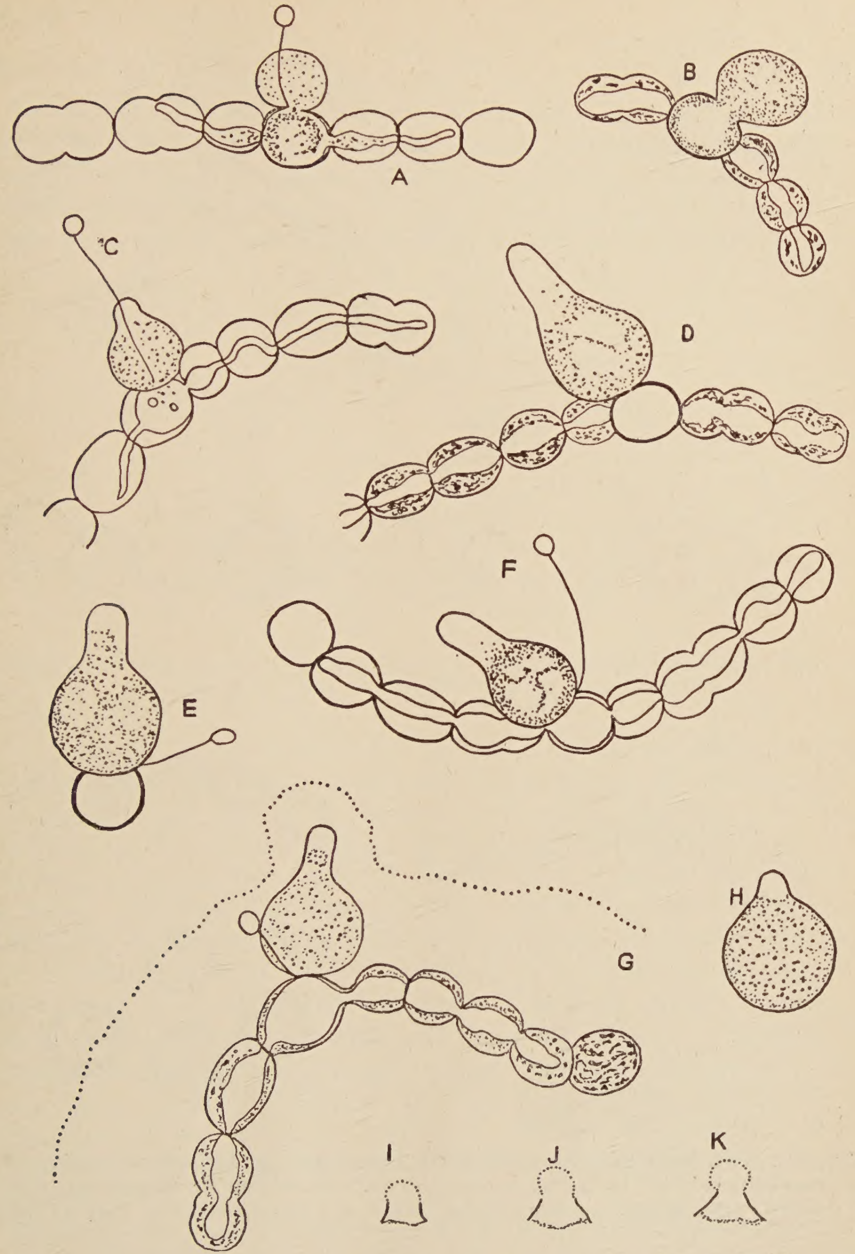
The protoplasm of the mature sporangium is granular with many bright minute globules; there are no large equally spaced oil globules such as characterize the mature sporangium of most chytrids. The mature sporangia vary in size from 9μ high by $6{\cdot}5\mu$ in diameter to 26μ high by 12μ in diameter.

The zoospore mass is clearly demarcated from the colourless apical portion of the sporangium (Text-fig. 2, H).

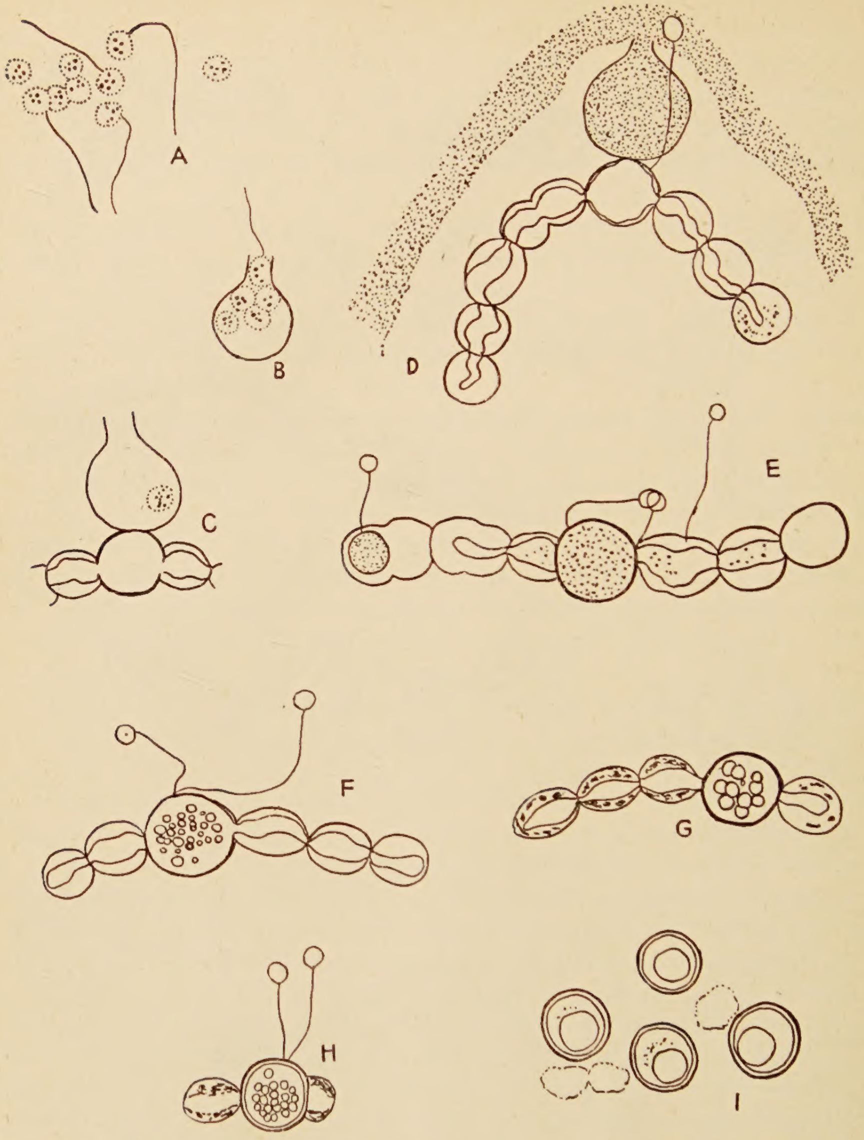
On dehiscence this colourless area gradually bulges, it becomes dome-shaped, and as it expands it becomes less dense and more difficult to observe; finally it is invisible. While this is happening the zoospore mass remains unaltered but suddenly it approaches the apex (when the colourless area is invisible) and the zoospores emerge rapidly one by one. Each zoospore rests for a few seconds just outside the sporangium before swimming away with a quick darting movement. The zoospore is spherical, 3μ in diameter, and contains about four bright granules in its cytoplasm (Text-fig. 3, A). The empty sporangium does not collapse after dehiscence.

If filaments of *Anabaena* bearing empty sporangia are mounted in indian ink the particles flow freely into the sporangia but never enter the prosporangium or rhizoids. It seems unlikely that the sporangium and prosporangium are separated by a septum, but rather that the connexion between the two is so narrow as to prevent the flow of particles into the prosporangium (Text-fig. 3, D). The host cell containing the prosporangium is always without visible contents and its wall often has a different optical property from that of neighbouring cells. It appears to be thicker, being lined by the cell wall of the parasite, and may consequently sometimes resemble a heterocyst (Pl. IX, Fig. A). After dehiscence the rhizoidal system becomes easily seen in living material, since it is represented by a clear area (usually occupying the central part of the cell), around which the dense remains of host protoplasm are aggregated. The empty encysted zoospore and its germ-tube are persistent (Pl. VIII, Fig. C). Eventually the host cells collapse and become colourless, thus contrasting strongly with the living portions of the filament.

The full story of resting-spore development is not quite clear. What seem to be early stages in the formation of resting spores have frequently been observed, and in a mass of dead *Anabaena* cells four examples were seen which may possibly be mature specimens (Text-fig. 3, I). The resting spore is indistinguishable from the prosporangium until a late stage in its development. Its content is at first granular, but as it matures it comes to contain up to thirty globules, roughly equal in size, which appear to be yellowish. The rhizoidal system in no way differs from that of the sporangium (Text-fig. 3, F, G).



Text-fig. 2, A–K. *Rhizosiphon crassum* Scherffel. A–F, development of the sporangium; note vacuolate protoplasm in D–F. G, H, mature sporangia. I–K, dehiscence; mucilaginous apex becomes more and more diffuse. A, C, F in acetocarmine; G in indian ink. (All × 1100.)

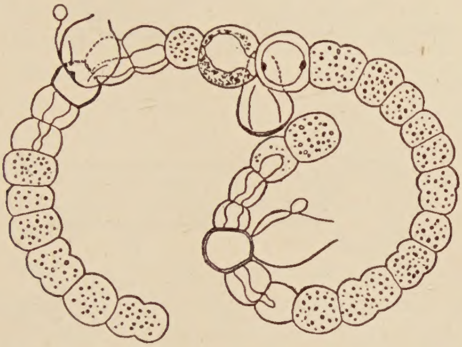


TEXT-FIG. 3, A–I. *Rhizosiphon crassum* Scherffel. A, zoospores. B, C, dehisced sporangia. D, empty sporangium mounted in indian ink showing entry of particles into the sporangium. E–H, immature resting spores. I, mature resting spores. E, F, in acetocarmine. (All × 1100.)

The wall of the mature spore is smooth and the content consists of a single large globule. The resting spore varies in size from 7 to 12 μ in diameter, and the cell in which it is contained usually appears larger than the neighbouring ones (Text-fig. 3, E–G). In some instances two empty zoospore cases (gametes?) and their germ-tubes have been seen attached to a cell containing a resting spore (Text-fig. 3, E, F, H), but as yet there is no definite evidence of a sexual process.

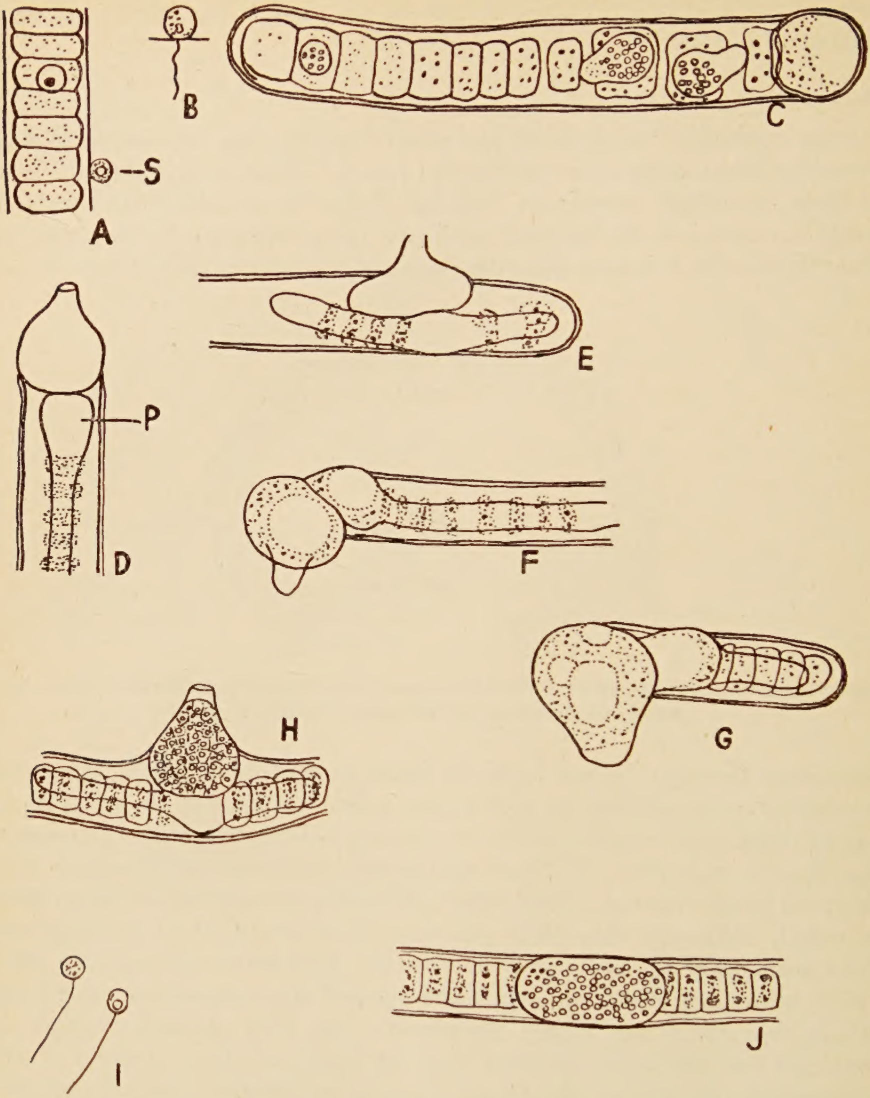
Discussion

In its method of development and general morphology this fungus closely resembles *Rhizosiphon crassum* Scherffel (1926). Although it is impossible to see from Scherffel's figures (see Text-fig. 5) that the content of the encysted zoospore passes into the host cell and forms a prosporangium at the distal end of the germ-tube, this method of development is made clear in the text. Other similarities between the two fungi are found in the tubular rhizoidal system, the flask-shaped sporangium with a clear apical portion and the presence of one or more large vacuoles in the protoplasm; of the two types of zoospore described by Scherffel only those with several small refractive granules were observed in my material. A few other differences between the two fungi must be noted. Although they both parasitize members of the Cyanophyceae, these hosts differ markedly in their habitats. *Filarszkya* occurred in a ditch which sometimes dried up, while the species of *Anabaena* on which my fungus was found are purely planktonic. The very marked changes in colour of the cells of *Filarszkya* and the death of those in parts of the trichome far distant from the fungal rhizoids were not a feature of my fungus. Again, the resting spore of *Rhizosiphon crassum* is apparently devoid of rhizoids. These differences do not seem sufficient to warrant the erection of a new species and for the present I identify it as *Rhizosiphon crassum* Scherffel.



TEXT-FIG. 4. Empty sporangia of *Rhizosiphon crassum* and *R. anabaenae* Rodhe et Skuja nov. comb. on *Anabaena* sp. Säbysjön, Sweden. (×800.)

Whiffen (1944) places *Rhizosiphon* in the subfamily Polyphagoideae Sparrow em. Whiffen which is characterized by the enlargement of the zoospore cyst into a prosporangium. However, as both Scherffel and I have observed that the prosporangium develops from the germ-tube of the zoospore, the position of *Rhizosiphon* in Whiffen's scheme is clearly in Diphophlyctoideae close to *Endocoenobium* Ingold.



Text-fig. 5, a–j. *Rhizosiphon crassum* Scherffel (1926). a, part of a filament of *Filarszkya*, by (*S*) a swarmer of the parasite which has come to rest. Above in a cell a spherical quite young intramatrical germling. The host cells are still quite healthy. b, germling which sends a thread-like rhizoid into an empty sheath of the host Cyanophyceae. c, larger round intramatrical germling. Cyanophyceae thread now appears as though it is impregnated with wine-red fluid. d, terminal, extra matrical empty zoosporangium, 'prosporangium' (*P*) grown out unilaterally. e, empty lateral zoosporangium; the watch-shaped orange-brown coloured remnants of the host cells are visible. f, zoosporangium with large central vacuole. g, zoosporangium with several large vacuoles, plasma dense white, refractive, and containing numerous scattered granules. At the apex the dense granular-free substance clearly demarcated towards the interior. h, the mature zoosporangium sits laterally on the prosporangium; opening already formed but swarmer mass still surrounded by thin membrane. i, two swarmers, one with solitary fat drop at the base of the flagellum, the other with numerous strongly refractive granules, fat drops? j, young oblong resting spore lying in host thread. (Legend translated from Scherffel, 1926.)

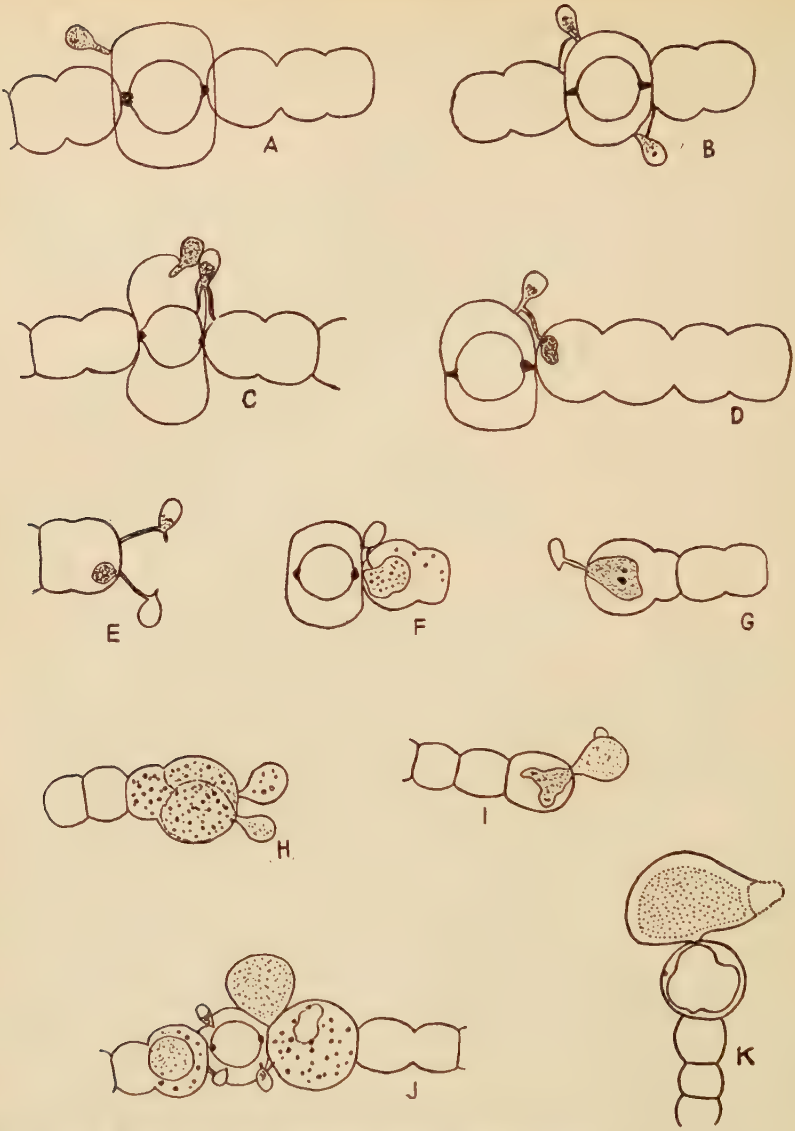
II. *RHIZOSIPHON ANABAENAE* RODHE ET SKUJA NOV. COMB.

Phlyctidium anabaenae was described by Skuja (1948) parasitizing *Anabaenae sphaerica* Born. et Flah., *A. spiroides* Kleb., and *A. macrospora* Kleb. from Sweden. Several aspects of its life-history puzzled me, namely, the exact method of development of the sporangium and the presence of so-called secondary sporangia (resting spores?). In November 1949 Dr. W. Rodhe of Uppsala sent me preserved material on which the description of this fungus was based. Further details of the life-history of *Phlyctidium anabaenae* are now known, and I much appreciate the kindness of Drs. W. Rodhe and H. Skuja for allowing me to describe this fungus more fully.

Anabaena cells infected by *P. anabaenae* are always located next to a heterocyst and are described by Skuja as young resting spores, but they seem to me to be vegetative algal cells, hypertrophied by the presence of the fungus so that they resemble young resting spores. The resting spores which I have observed in both healthy and infected filaments are long, oval, and nearly always occur between vegetative cells rather than adjacent to a heterocyst.

Although Skuja states that the fungal infection is by way of a heterocyst, the precise method of development of the sporangium is not clear. After examination of the material I found that this fungus has a very similar life-history to that already described for *Rhizosiphon crassum*. However, there are several interesting differences. Whereas the zoospore in *R. crassum* encysts on, or just within, the mucilage of the algal filament and produces a fine thread to a vegetative cell, the zoospore of *Phlyctidium anabaenae* always settles on a heterocyst. It then produces a short, lateral thread to an adjacent host cell which at that time is indistinguishable from any other vegetative cell in the filament (Pl. II, Fig. B, and Text-fig. 6, B, C). The content of the encysted zoospore gradually passes into the host cell. By the staining technique described on page 130, early stages in the growth of the fungus can be seen. Within the infected cell the fungus body (prosporangium) enlarges; at the same time the algal cell usually increases in size (Text-fig. 6, G). The prosporangium is spherical, 5–11 μ in diameter, and never occupies the whole volume of the cell in which it is embedded. No rhizoidal system has been observed connected with the prosporangium, nor do adjacent cells appear to be diseased. Three prosporangia have been found in a single host cell. The sporangium is formed as a direct outgrowth from the prosporangium. It is always produced on the side of the host cell nearest to the heterocyst, and probably makes its way through or adjacent to the place where the lateral tube from the zoospore pierced the algal wall.

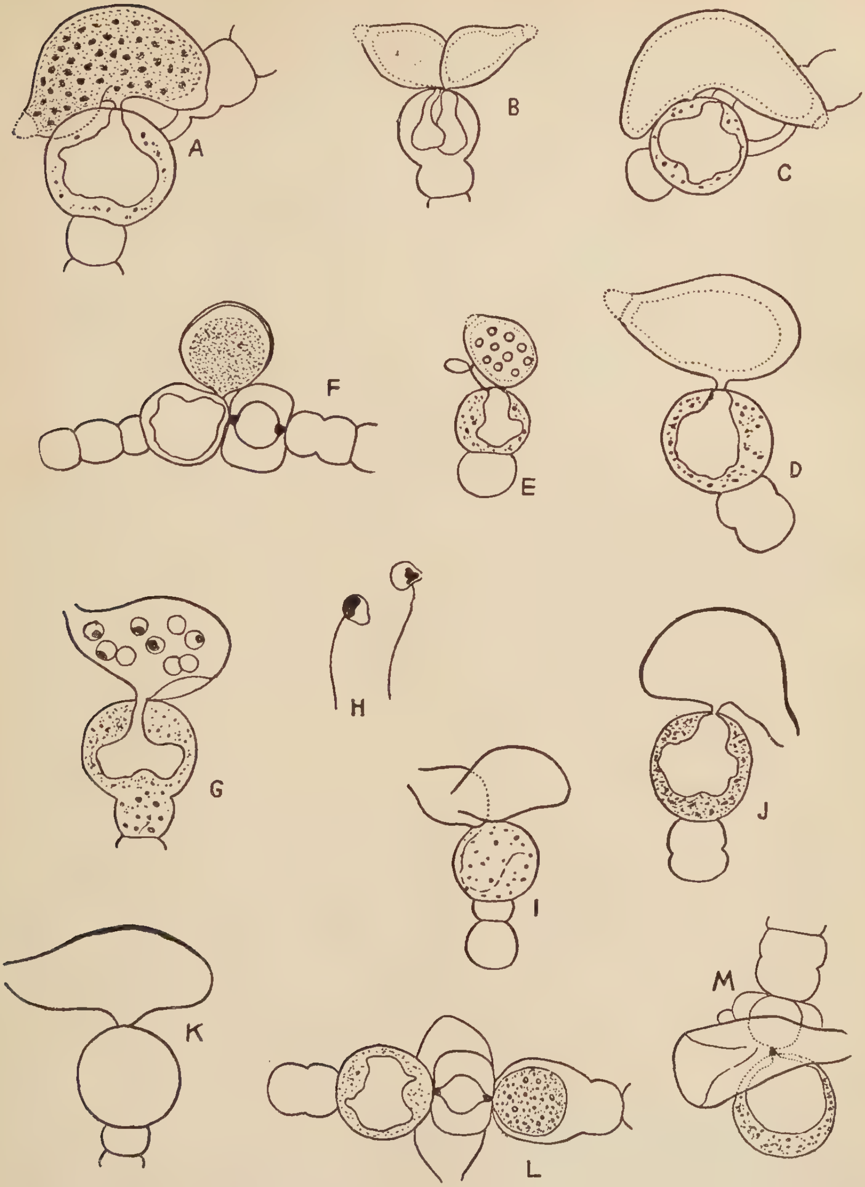
The sporangium begins its development as a minute bud with hyaline content (Text-fig. 6, H). This grows into a pear-, sack-, or retort-shaped sporangium. The last two types may develop somewhat laterally, and one side becomes drawn out to form a dehiscence papilla. In such sporangia, which are usually the largest, the papilla is set at right angles to the length



Text-fig. 6, A–K. *Rhizosiphon anabaenae* nov. comb. A, encysted zoospore attached to heterocyst of *Anabaena*. B–C, production of lateral germ-tube to a neighbouring host cell. D–G, passage of content into the host cell and early stages in the formation of the prosporangium. H–J, bud-like outgrowths which will develop into sporangia. K, empty prosporangium and immature sporangium with mucilaginous apex. The heterocyst in E, G–I, has become detached from the rest of the algal filament. (A–F, × 1100; G–K, × 940.)

of the algal filament (Text-fig. 6, K). The mature sporangium appears to have a thickened mucilaginous apex and is therefore presumed to be inoperculate.

The retort- and sack-shaped sporangia vary in size from 8 to 14 μ high by

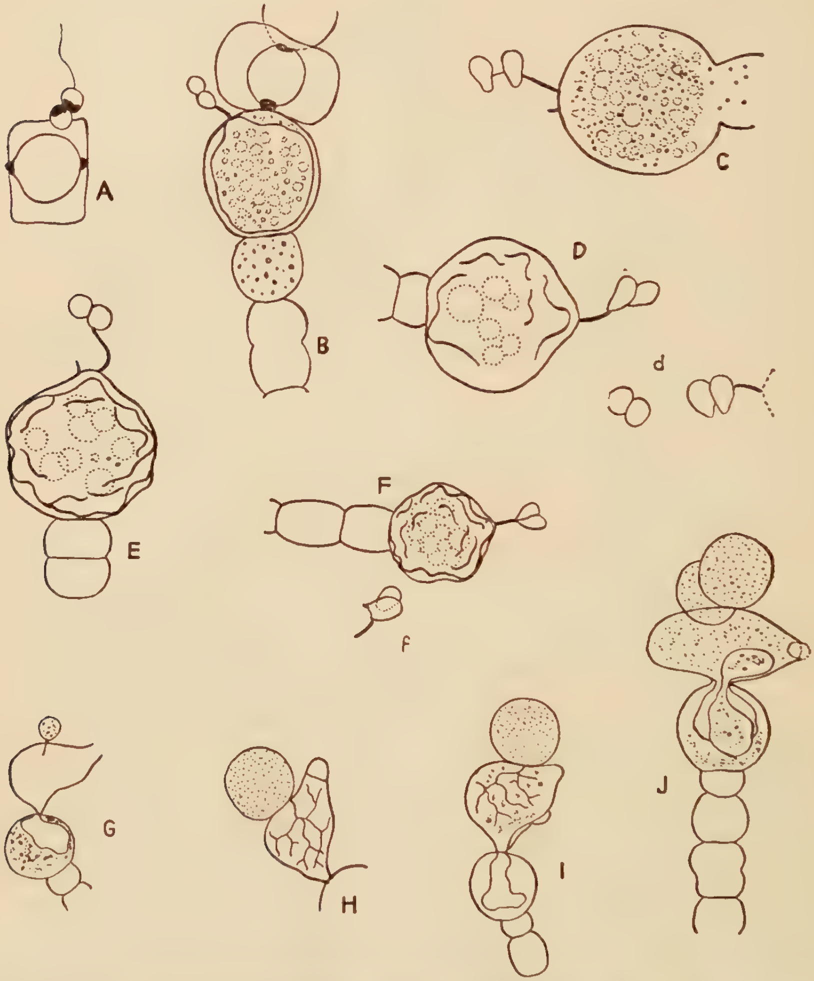


Text-fig. 7, a–m. *Rhizosiphon anabaenae* nov. comb. a–e, variation in sporangial size and form. f, sporangium in end view. g, sporangium with zoospores. h, zoospores. i–m, empty sporangia. (H, × 1100; rest × 940.)

18–27 μ long, while the smaller pear-shaped forms range from 8 to 13 μ high by 4·6–8·5 μ in diameter.

Zoospores were not observed by Skuja and I observed them only once in the preserved material (Text-fig. 7, h). The sporangium does not collapse after dehiscence, but the empty prosporangium no longer retains its spherical nature, instead it becomes irregularly undulate (Text-fig. 7, D, G, J).

Regarding the *sekundär zoosporangien* or ?*Dauerzellen* it seems fairly clear



Text-fig. 8, A–J. *Rhizosiphon anabaenae* nov. comb. A, possible early stage in fusion of gametes. B, C, young resting spores with adherent gametes. D–F, mature resting spores; in D and F fused gametes are shown in diverse views *d f*. G, zoospore of hyperparasite on empty host sporangium. H–J, sporangia of hyperparasite; rhizoidal system visible in H and I. (A, C–E, *d f* ×1100; rest ×940.)

that these bodies are sporangia of a hyperparasite. A branched rhizoidal system was found attached to these sporangia in infected cells of *Phlyctidium anabaenae*. No resting spores of the hyperparasite were found, and further observations on living material are necessary to determine its systematic position.

The true resting spores of *P. anabaenae* are found within the algal cells and are formed from the endobiotic swelling (prosporangium). The lateral thread connecting the zoospore to the resting spore is noticeably thicker than that formed in association with a prosporangium (Text-fig. 8, C–F). After much searching it became fairly clear that a sexual process is involved. Fusion appears to take place isogamously, between two gametes. What is possibly a very early stage in fusion is seen in Text-fig. 8, A. It seems that the gametes may make contact directly or laterally (as shown in Text-fig. 8, C) by means of a short tube. The young resting spore itself differs in no way from a young prosporangium, but it enlarges together with the host cell in which it is embedded to a much greater size. The mature resting spore is spherical to subspherical (10–17 μ in diameter) and possesses a thick, smooth, yellowish wall which is somewhat undulate (Text-fig. 8, D–F). The content consists of several large globules. A prosporangium and resting spore may occupy the same host cell, but usually a cell contains only one resting spore.

Phlyctidium anabaenae exhibits endoexogenous development of a type similar to that described for *Rhizosiphon crassum*. It is clear that in spite of the so-called sac-like rhizoid (which is actually a prosporangium) the fungus cannot be placed in the genus *Phlyctidium*. It is decided to transfer it to *Rhizosiphon* where a similar type of life-history exists; the name therefore becomes *R. anabaenae* Rodhe et Skuja nov. comb. An emended but necessarily incomplete diagnosis is given.

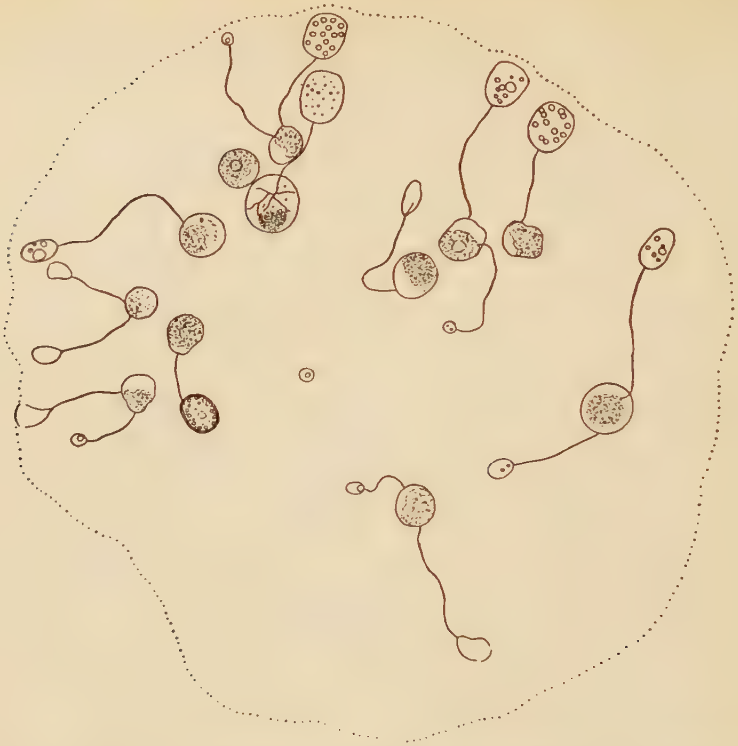
Rhizosiphon anabaenae Rodhe et Skuja nov. comb.

Endobiotic prosporangium spherical, 5–11 μ in diameter, arising as a swelling from the tip of the germ-tube of the zoospore. Sporangium pear- (8–13 μ high by 4·6–8·5 μ in diameter), sack-, or retort-shaped (8–14 μ high by 18–27 μ long) with a mucilaginous dehiscence papilla. Resting-spore formation preceded by fusion of isogamous gametes, one of which has previously come to rest and ?germinated. Resting spore endobiotic, spherical to subspherical, 10–17 μ in diameter, with a thick, smooth, yellowish undulate wall; content consisting of several large globules.

Parasitic and causing hypertrophy of *Anabaena* cells in the plankton of Säbysjön, Sweden.

Rhizosiphon anabaenae Rodhe et Skuja nov. comb.

Endobiotisches Prosporangium kugelförmig von 5–11 μ Durchmesser, erscheint als eine Anschwellung auf der Spitze des Zoosporenkeimschlauches. Sporangiumbirnen- (8–13 μ hoch × 4·6–8·5 μ Durchmesser), kolben- oder sackförmig (8–14 μ hoch × 18–27 μ lang) mit einer schleimigen apikalen Papille. Dauersporenbildung geschieht nach der Verschmelzung von Isogameten von denen einer früher zur Ruhe gekommen ist und ?gekeimt hat. Dauerspore endobiotisch, kugelig oder kurz ellipsoidisch (10–17 μ Durchmesser), mit einer dicken glatten gelben wellenförmigen Wand; Inhalt besteht aus mehreren grossen Tröpfchen.



TEXT-FIG. 9. A colony of *Gemellicystis neglecta* the cells of which are heavily infected by *Rhizophidium fulgens* n.sp. M, mucilage. (× 700.)

Schmarotzend und bewirkt Hypertrophie bei *Anabaena*-zellen im Plankton des Säbysjön, Schweden.

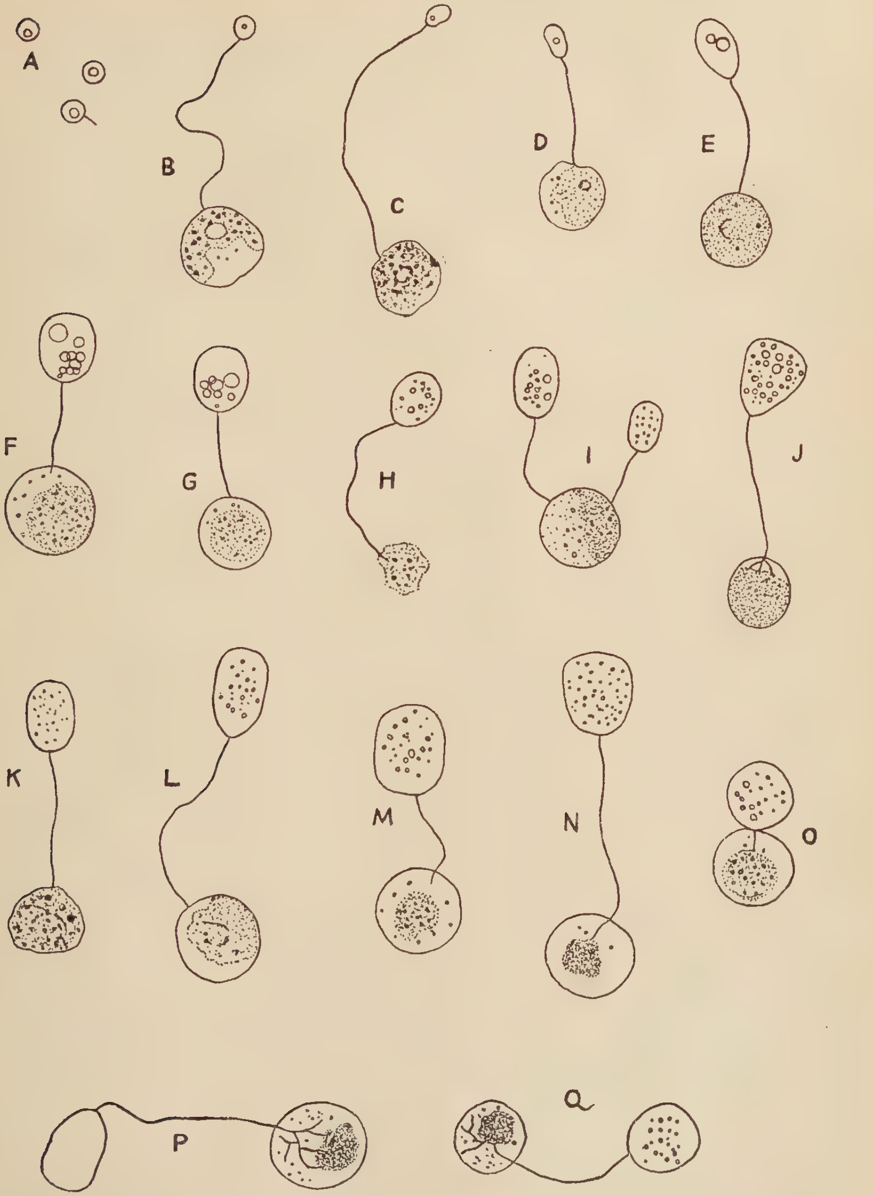
III. *RHIZOPHIDIUM FULGENS* N.SP.

TABLE I

Occurrence on Gemellicystis neglecta (*Teiling*) *Skuja*

Lake.	1949.	1950.
Loweswater	May 6–18	Apr. 11
Crummock Water . . .	May 6	May 10
Swithland Reservoir . . .	—	Apr. 26

This fungus is parasitic on the colonial green alga *Gemellicystis neglecta* (Teiling) Skuja (Text-fig. 9 and Pl. XI, Fig. A). The spherical encysted zoospore produces a fine thread or germ-tube which grows through the mucilage until it reaches a host cell (Text-fig. 10, A, B). This germ-tube varies greatly in length (up to 70μ long). Only one specimen devoid of a germ-tube and thus sessile on the algal cell was encountered (Text-fig. 10, O). Having made contact with a host cell the zoospore enlarges into an oval or irregularly oval sporangium the apex of which is often dome-shaped and



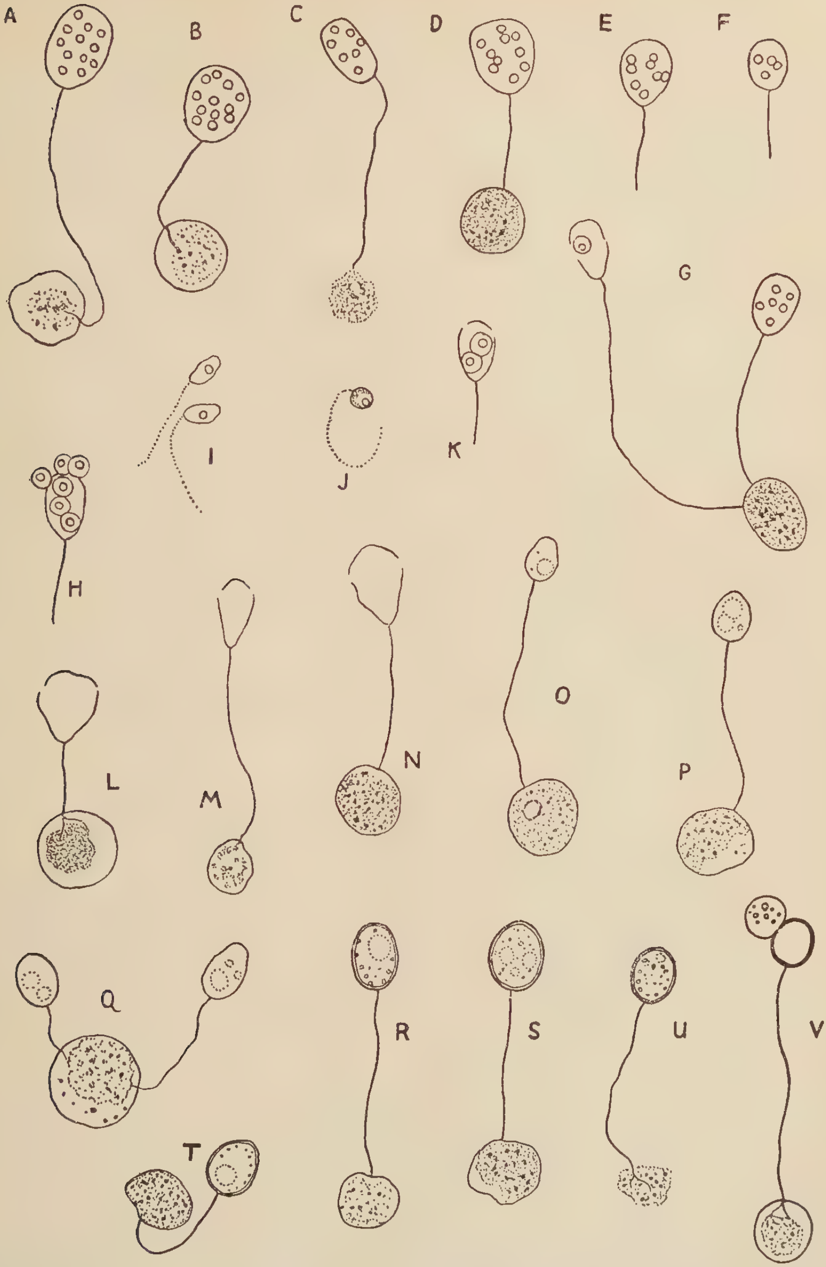
Text-fig. 10, A–Q. *Rhizophidium fulgens* n.sp. A, encysted zoospores; one has begun to produce a germ thread. B, zoospore which has made contact with a host cell. C–N, enlargement of the zoospore and formation of sporangia. O, sporangium which is sessile on a host cell. P, Q, endobiotic rhizoidal system clearly visible; (P) stained in iodine in potassium iodide. (All × 1100.)

broader than the base. The endobiotic rhizoidal system is difficult to see owing to the dense chloroplast of the alga. However, a few short branches arising from the germ-tube have been distinguished (Text-fig. 10, P, Q). As the chytrid grows the host content becomes disorganized and a colourless area often develops near the point of entry of the germ-tube; the remainder of the cell retains its green colour. Eventually the cell wall may become invisible and the entire chloroplast become colourless or be changed into a small brown mass. The wall of the mature sporangium is delicate and the germ-tube thickens slightly if at all. Mature sporangia vary in size from 6 μ high by 4·5 μ broad to 10 μ high by 8 μ broad and contain from 4 to 18 refractive globules, each indicating the position of a zoospore (Text-fig. 11, A–G). Liberation of the zoospores takes place through two pores formed in the apical corners of the sporangium wall (Text-fig. 11, L–N). The zoospores emerge very slowly one by one through the openings and it takes some time for the sporangium to empty. The zoospore (2·5 μ in diameter) rests for a few minutes before swimming away with a smooth gliding movement. While resting it may assume an oval shape and its apex be somewhat metabolic. The zoospore has an anterior refractive globule, and at the point of insertion of the posterior flagellum the protoplasm appears denser (Text-fig. 11, J). After dehiscence the empty sporangium collapses, and the two dehiscence pores become difficult to see.

The resting spore is formed asexually and when young is distinguishable from a sporangium by its narrowed apex and by the presence of one or two large refractive globules (Text-fig. 11, O–Q). When mature, the resting spore is oval and varies in size from 6 μ high by 4·5 μ broad to 8 μ high by 6 μ broad. The thickened wall remains smooth and colourless, the oily content consists of numerous small peripheral globules and one or two larger, more central ones (Text-fig. 11, R–U). On germination the resting spore functions as a prosporangium (Text-fig. 11, V). The zoospores produced as a result of germination have not been observed. In its habit this fungus resembles *Rhizophidium sphaerocystidis* Canter which likewise infects a planktonic alga surrounded by a wide mucilage envelope. In both fungi the sporangium develops from the encysted zoospore while the germ-tube remains as a stalk-like portion. However, in the method of dehiscence and in resting-spore formation these fungi differ greatly. The writer considers the fungus on *Gemellicystis* to be specifically distinct and suggests the name *Rhizophidium fulgens.*

Rhizophidium fulgens n.sp.

Thallus monocentric eucarpic, consisting of a sporangium developed from the encysted zoospore, an unswollen stalk-like region within the mucilage envelope of the alga, and a branched rhizoidal system inside the host cell. The sporangium varies in shape from obovate to oval (6 μ high × 4·5 μ broad to 10 μ high × 8 μ broad) and contains from 4 to 18 zoospores. The zoospores (2·5 μ in diameter) are anteriorly uniguttulate and posteriorly uniflagellate;



TEXT-FIG. 11, A–V. *Rhizophidium fulgens* n.sp. A–G, mature sporangia. H, dehiscing sporangium. I, metabolic zoospores. J, free-swimming zoospore. K, incompletely dehisced sporangium. L–N, empty sporangia. O–Q, young resting spores. R–U, mature resting spores. V, germinated resting spore. (All × 1100.)

they escape from the sporangium via two pores formed in the apical corners of the wall. Resting spore asexually formed, oval in shape (6 μ high × 4·5 μ broad to 8 μ high × 6 μ broad) with a smooth, thick, colourless wall. The content contains numerous small peripheral refractive globules and one or two larger globules. On germination functioning as a prosporangium. Parasitic on *Gemellicystis neglecta* (Teiling) Skuja in Loweswater and Crummock Water, the English Lake District, and Swithland Reservoir, Leicestershire.

Rhizophidium fulgens n.sp.

Thallus bestehend aus einem Sporangium (aus einer eingekapselten Zoospore entwickelt), aus einem nicht geschwollenen stengelförmigen Teil innerhalb der Schleimhülle der Alge, und aus einem verzweigten Rhizoidsystem innerhalb der Wirtzelle. Das Sporangium wechselt in Form von verkehrt eiförmig bis oval (6–10 μ hoch × 4·5–8 μ breit) und enthält von 4 bis 18 Zoosporen. Die Zoosporen (2·5 μ Durchmesser) besitzen vorne nur ein Öltröpfchen und hinten ein Flagellum; sie entschlüpfen dem Sporangium durch zwei Poren, welche in den gipfelständigen Wandecken gebildet sind. Dauerspore asexuell gebildet und oval in Form (6–8 μ hoch × 4·5–6 μ breit) mit einer glatten dicken farblosen Wand. Der Inhalt weist zahlreiche kleine randständige lichtbrechende Tröpfchen und ein oder zwei grössere Tröpfchen auf. Funktioniert bei Keimung als Prosporangium.

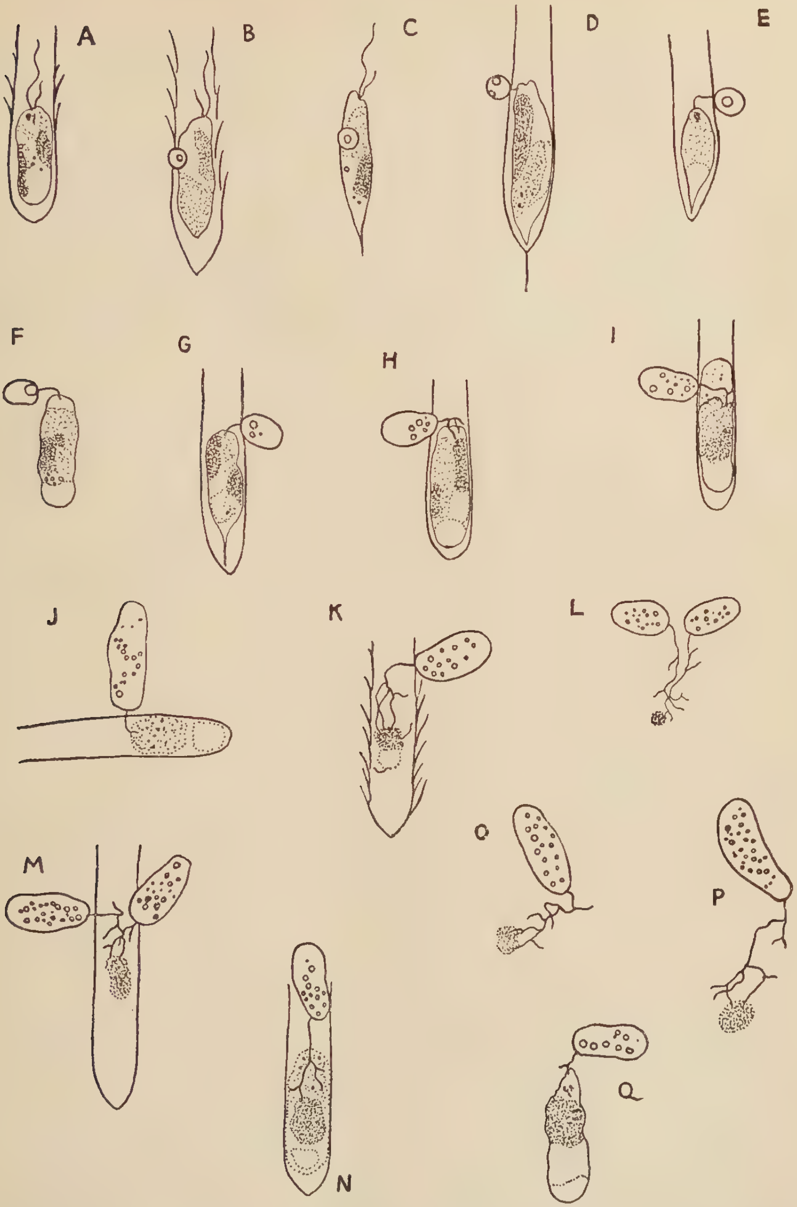
Schmarotzend auf *Gemellicystis neglecta* (Teiling) Skuja im Loweswater und Crummock Water, Englischen Lake District, und im Stausee 'Swithland', Leicestershire, England.

IV. *RHIZOPHIDIUM HYALOBRYONIS* N.SP.

This fungus was observed in Loweswater, the English Lake District, during April 1949 and 1950 as a parasite of *Hyalobryon mucicola*[1] Pascher. *Hyalobryon* lives attached to other algae in the plankton and in this instance was found on *Coelosphaerium Nagelianum* Kütz. The protoplast of the alga is enclosed in a delicate envelope the exact form of which is difficult to observe unless stained with Ruthenium Red. *Hyalobryon* is a delicate alga which is difficult to keep alive in the laboratory and the protoplast rapidly disintegrates on being mounted under a coverslip.

A zoospore of the fungus, containing a single refractive globule, settles on the outer surface of the *Hyalobryon* envelope (Text-fig. 12, B) and sends a delicate rhizoid into the host cell (Text-fig. 12, E–G). At this stage the alga appears to be healthy and its flagella are still active. Thus the fungus may be considered as a parasite. The zoospore gradually enlarges into a cylindrical sporangium which is often set at right angles to the length of the host envelope (Text-fig. 12, G–K). The protoplasmic changes in the sporangium leading to the formation of a number of refractive globules of equal size are similar to those described for most chytrids. The mature sporangia contain from 6 to 18 globules each indicating the position of a zoospore (Text-fig. 13, A–F). The

[1] Tentatively assigned to this species pending further investigation by Dr. J. W. G. Lund.



TEXT-FIG. 12, A–Q. *Rhizophidium hyalobryonis* n.sp. A, a healthy algal cell in its envelope. B, C, encysted zoospores. D–Q, enlargement of zoospore and stages in the development of the cylindrical sporangium. The rhizoidal system is clearly seen in K–P. In figs. C, F, L, O–Q it was impossible to distinguish the envelope surrounding the *Hyalobryon* cells. (All × 1100.)

sporangia range in size from 4·5 μ high by 2·5 μ broad to 15·5 μ high by 5 μ broad.

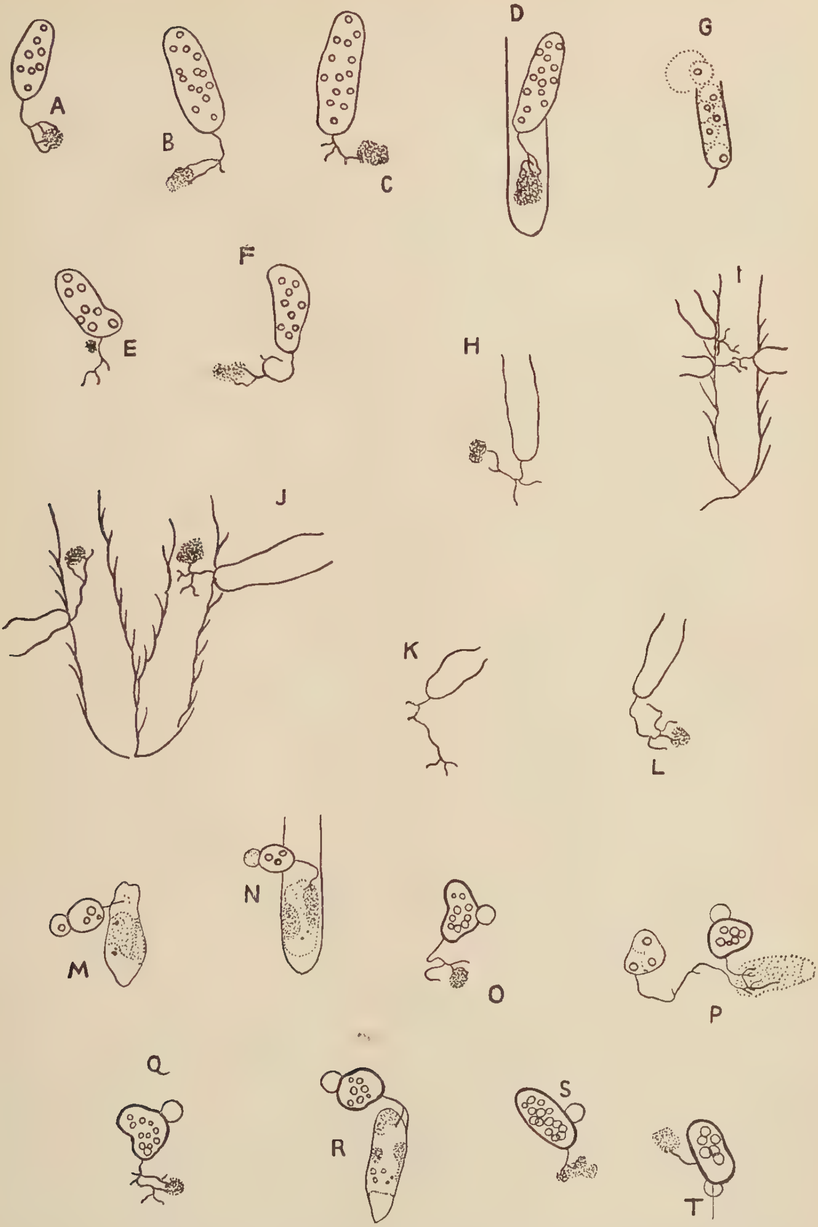
Up to three sporangia have been found on a single algal cell. The rhizoidal system of the fungus is clearly visible. It consists of a short thread which branches within the algal cell (Text-fig. 12, K–P). As the sporangium develops the host content becomes disorganized and finally only a small brown residue remains (Text-fig. 13, J). On dehiscence the apex of the sporangium dissolves (no papilla is formed) and the zoospores emerge slowly. They rest for a few seconds at the orifice before swimming away. The zoospore (2·5–3 μ diameter) has a large oil globule and single posterior flagellum. The wall of the empty sporangium retains its shape and remains clearly visible (Text-fig. 13, H–L).

The resting spore is formed after sexual fusion. The earliest stage I observed is shown in Text-fig. 13, M. A small male cell 2–3 μ diameter (resembling an encysted zoospore) settles on a larger female cell. The content of the male passes into the female and the latter becomes surrounded by a thick, colourless, smooth wall. The mature resting spore (Text-fig. 13, O–T) differs slightly in shape from a sporangium; it is usually less elongate and may even be oval or bean-shaped. The content is composed of several small refractive globules, and it possesses a rhizoidal system similar to that described for the sporangium. The resting spore varies in size from 5·5 μ high by 4 μ broad to 9·5 μ high by 4·5 μ broad (one very small specimen measured 4·5 μ high by 3 μ broad).

Since the sporangium is cylindrical in shape and in the majority of specimens its long axis is at right angles to the envelope of the host, it resembles species of *Rhizophidium* in Sparrow (1943, p. 162) grouped under Section V. However, it cannot be identified with any of the existing species. Again, it is unlike *R. chrysopyxidis* Scherffel, the only other chytrid recorded on a member of the Chrysophyceae. The type of sexual fusion exhibited by the fungus under consideration has now been reported in many species of *Rhizophidium*. It is, however, considered to be a new species and the binomial *R. hyalobryonis* is suggested.

Rhizophidium hyalobryonis n.sp.

Thallus monocentric consisting of a sporangium and branched rhizoidal system. Sporangium cylindrical, 4·5 μ high × 2·5 μ broad to 15·5 μ high × 5·0 μ broad, usually with its long axis set at right angles to that of the host envelope. On dehiscence the apex of the sporangium dissolves and from 6 to 18 zoospores emerge. Zoospore spherical, 2·5–3 μ in diameter with a refractive globule and single posterior flagellum. Resting spore cylindrical, oval, or bean-shaped, 4·5 μ high × 3 μ broad to 9·5 μ high × 4·5 μ broad; wall, smooth, colourless, content composed of several small refractive globules. The resting spore is formed after fusion of the content of a small male 2·3 μ diameter, with a larger female cell. The empty male cell remains adherent to the mature resting spore. Germination not observed. Parasitic on *Hyalobryon mucicola* Pasher, Loweswater, the Lake District, England.



TEXT-FIG. 13, A–T. *Rhizophidium hyalobryonis* n.sp. A–F, mature sporangia. G, dehiscence. H–L, empty sporangia. M, conjugating thalli. N, male thallus still retains some content, but oil globule has disappeared. O–T, mature resting spores each with an empty adherent male thallus. (All × 1100.)

Rhizophidium hyalobryonis n.sp.

Thallus aus einem Sporangium und einem verzweigten Rhizoidsystem bestehend. Sporangium zylindrisch (4·5–15·5 μ hoch × 2·5–5 μ breit) gewöhnlich mit der längeren Achse senkrecht zu derjenigen der Hüllen der Wirtzelle. Bei der Entleerung löst sich die Spitze des Sporangiums auf und es kommen von 6–18 Zoosporen heraus. Zoospore kugelförmig von 2·5–3 μ Durchmesser mit einem lichtbrechenden Tröpfchen und einem einzigen hinteren Flagellum. Dauerspore zylindrisch oval oder bohnenförmig (4·5–9·5 μ hoch × 3–4·5 μ breit), Wand glatt farblos, Inhalt bestehend aus mehreren kleinen lichtbrechenden Tröpfchen. Die Dauerspore wird nach Verschmelzung des Inhalts einer kleinen männlichen (2–3 μ Durchmesser) und einer grösseren weiblichen Zelle gebildet. Die leere männliche Zelle bleibt an der reifen Zoospore klebend. Keimung nicht beobachtet.

Schmarotzend auf *Hyalobryon mucicola* Pascher im Loweswaterem, Englischen Lake District.

V. *CHYTRIOMYCES TABELLARIAE* (SCHRÖTER) CANTER

TABLE II

Occurrence of C. tabellariae *in the English Lake District based on samples collected at approximately monthly intervals since January 1949.*

Lake.	1949.	1950.	Host.
Crummock Water	Apr. 7	Apr. 5–May 31	*Tabellaria* sp.
Derwent Water	Jan. 13–Mar. 7	—	,,
Bassenthwaite	Jan. 13–Feb. 10	—	,,
Thirlmere	Feb. 10–June 1	Feb. 9–May 3	,,
Hawes Water	Apr. 25	—	,,
Elterwater	Mar. 17	—	*T. fenestrata* var. *intermedia* Grün.
Coniston Water	—	Feb. 22	*T. flocculosa* (Roth.) Kützing
Brothers Water	—	Jan. 4	,, ,,
Ullswater	Feb. 3	—	,, ,,

The structure and life-history of this fungus have already been described (Canter, 1949) from specimens parasitizing *Tabellaria flocculosa* (Roth.) Kützing in a mud pool near Blelham Tarn, Lancashire. In the same paper three records of its occurrence in the plankton were noted, on *Tabellaria fenestrata* (Lyngbye) Kützing in Crummock Water, Bassenthwaite, and Buttermere. Miss B. Knudson of the Freshwater Biological Association, who is working on the morphology of *Tabellaria*, tells me that the diatom host on these occasions was not the *T. fenestrata* of Lyngbye and pending further investigation it is better designated as *Tabellaria* sp. As a result of the 1949–50 Lake District survey *Chytriomyces tabellariae* appears to be widespread on species of *Tabellaria* found in the plankton (see Table II). Also it was found on *T. flocculosa* in an isolated plankton sample collected from Moss Eccles Tarn, Claife Heights, near Hawkshead, Lancashire, January 12, 1950. It has not yet been seen in the Lake District on *Tabellaria fenestrata* senu stricto or

T. fenestrata var. *asterionelloides* Grün. The fungus is illustrated in Text-fig. 14, A–P. In structure development and resting-spore formation it is identical with the material described by Canter (1949). The rhizoidal system, however, was much less difficult to observe (Text-fig. 14, H). A few insignificant differences were noted. In some specimens the sporangium possessed an extramatrical germ-tube which bifurcated (Text-fig. 14, F). There was no evidence to show that both threads entered a host cell. Again, some very small sporangia which could have only produced one or two zoospores were seen. The resting spores were similar in structure to those shown in Canter (1949, Text-fig. 2 *j*, *k*).

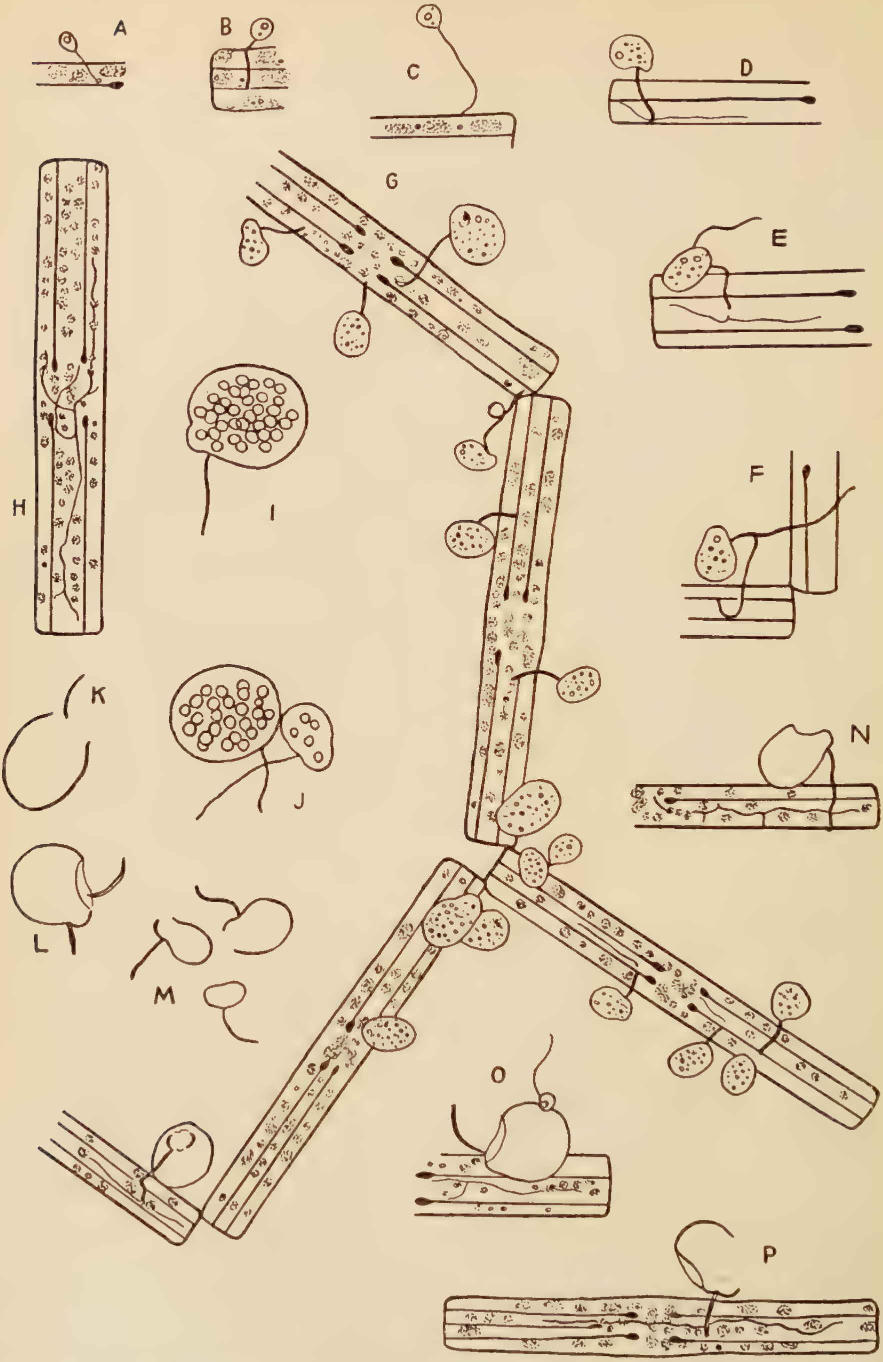
VI. *AMPHICYPELLUS ELEGANS* INGOLD

Since *Amphicypellus elegans* was described by Ingold (1944) from Windermere, further observations on its life-history from living material have been made, the details of which will be published later. The writer has also examined numerous occasional, preserved samples of plankton containing *Ceratium hirundinella* collected and sent to her by other workers. As a result several new localities are given for *Amphicypellus elegans* (see Table V). From 1947 to 1949 weekly samples from Windermere, North and South Basins, Blelham Tarn, and Esthwaite Water have been examined for the presence of this fungus. The majority of specimens occur on *Ceratium hirundinella* O.F.M., but it may also infect *Peridinium cinctum* Ehrenb. (cf. Tables III and IV). Records prior to 1947 represent casual observations only. The earliest record of *Amphicypellus elegans* is in a preserved sample (West collection) from Bowness, Windermere, September 1903. The 1949 Lake District Survey has shown it to be present in five other lakes.

TABLE III

Occurrence of Amphicypellus elegans *on* Ceratium hirundinella *O.F.M. in the Lake District*

Lake.	1945.	1946.	1947.	1948.	1949.
Windermere: South Basin	—	—	July 4–Oct. 14	June 8–Oct. 26	June 29–Aug. 9
Windermere: North Basin	July 31, Sept. 3	Aug. 21	July 8–Oct. 27	June 7–Nov. 1	June 28–Sept. 6
Esthwaite Water	—	June 26–Sept. 24	July 10–Oct. 16	July 12–Sept. 29	June 26–Oct. 17
Blelham Tarn	—	—	July 17–Sept. 3	June 22–Sept. 16	July 19–Sept. 12
Thirlmere	—	—	—	—	Sept. 21
Loughrigg Tarn	—	—	—	—	Aug. 3–Nov. 22
Loweswater	—	—	—	—	June 1–Oct. 3
Ullswater, East	—	—	—	—	Sept. 14
Ullswater, West	—	—	—	—	Sept. 14



TEXT-FIG. 14, A–P. *Chytriomyces tabellariae* (Schröter) Canter. A, an encysted zoospore. B–F, enlargement of the zoospore and formation of young sporangia. G, four heavily infected cells of *Tabellaria*. H, endobiotic rhizoidal system. I, J, mature sporangia. K–P, empty sporangia. (I, J × 1070; rest × 746.)

TABLE IV

Occurrence of Amphicypellus elegans *on* Peridinium cinctum *Ehrenb. in the Lake District*

Lake.	1948.	1949.
Windermere: South Basin	June 30, Oct. 5	Sept. 5
Windermere: North Basin	June 7–Sept. 20	Sept. 13, Oct. 11
Blelham Tarn	July 5	—
Esthwaite Water	—	July 12–Oct. 3
Loweswater	—	June 1, Sept. 7–21
Thirlmere	—	Sept. 21

TABLE V

Records of Amphicypellus elegans *on* Ceratium hirundinella *O.F.M. outside the Lake District*

Country.	Lake.	Actual date when sample was collected.	
England	Rostherne Mere	—	1912
	Knutsford Mere	Aug.	1922
Scotland	Loch Lomond	Aug. 3	1949
Denmark	Almindsø	July 19	1949
	Furesø	Aug. 20	1949
Sweden	Valloxen	July 25	1945
	Erken	Aug. 3	1945
Italy	Lago Lugano	Nov. 3	1945
		May 12	1946
	Lago Como	June 28	1946
	Lago Mergozzo	Sept. 24	1948
		July 29, Nov. 3	1949
	Lago Maggiore	Nov. 17	1948
		June 28, Sept. 26	1949

VII. *DANGEARDIA MAMMILLATA* SCHRÖDER

D. mammillata has already been recorded in England by Canter (1946) from Barn Elms Reservoir, London, and by Ingold (1940) from Swithland Reservoir, Leicestershire. The writer has received living plankton samples from the latter locality since January 1950 and found this fungus present from February 28 to April 26. New records are from the London Metropolitan Water Board Reservoirs; Walthamstow Race Course, March 30, 1949, and King George (Lea), May 7, 1949; the host in both instances was *Eudorina elegans* Ehrenb.

Dr. B. M. Griffiths (1925) noticed a fungus attacking *E. elegans* from Newton Mere, Cheshire, in August 1922. I have examined this sample and there is no doubt that the fungus concerned is *D. mammillata*. Although *Eudorina elegans* is widespread in the Lake District and is infected by fungi, *D. mammillata* appears to be absent from this locality.

VIII. *RHIZIDIUM WINDERMERENSE* CANTER

This fungus was described by Canter (1950) and a table showing its occurrence in certain lakes was given from 1947 to May 1949. The completed records for 1949 in three lakes, which are sampled once a week, are seen in Table VI. At the time of writing (June 1950) it will be seen that *R. Windermerense* has already reappeared in Windermere, North and South Basins. New records are from Bassenthwaite in the Lake District, June 1, September 21, 1949, and from Lago Lugano, Italy (preserved samples), collected on July 20, 1946, 1949.

TABLE VI

Occurrence of R. Windermerense *on* Gemellicystis neglecta (*Teiling*) *Skuja*

Lake.	1947.	1948.	1949.	Records to June 1, 1950.
Windermere: North Basin	June 15–Nov. 17	Aug. 30–Oct. 11	Apr. 19–May 9 Aug. 9–Oct. 11	May 2–
Windermere: South Basin	June 11–Aug. 5 Oct. 14	Sept. 8–Oct. 26	Apr. 28–Sept. 19	May 15–
Esthwaite Water	June 13–July 10	July 12–23	June 15–Sept. 12	

IX. *ZYGORHIZIDIUM MELOSIRAE* CANTER

Table VII gives a completed picture of the dates of occurrence of this fungus during 1949 and up to the time of disappearance of its host in the spring of 1950. For earlier records see Canter (1950). Outside the Lake District it has been observed in preserved samples (R. Southern and A. C. Gardiner collection) from Lough Derg, Ireland, collected March 14, 1921, and March 29, 1922.

TABLE VII

Occurrence of Z. melosirae *on* Melosira italica (*Ehrenb.*) *Kütz.*

Lake.	1949.	1950.
Esthwaite Water	Feb. 15–Apr. 27	Jan. 9–May 15
Blelham Tarn	Jan. 12–May 9 Nov. 21	Jan. 2–May 8
Windermere: South Basin . . .	Mar. 23–Apr. 20	Mar. 12–Apr. 4
Windermere: North Basin . . .	Jan. 24–May 9	Feb. 21–Mar. 12
Hawes Water	Feb. 3–Apr. 7 Nov. 21–Dec. 4	Jan. 3–Mar. 13
Loughrigg Tarn	Mar. 21–May 6 Oct. 28–Dec. 21	Jan. 18–Mar. 15
Ullswater, East	Feb. 3–Apr. 28	
Ullswater, West	Mar. 3–May 23	
Loweswater	Apr. 7–May 6	

X. *ZYGORHIZIDIUM PARVUM* CANTER

Since Canter (1950) further records of the occurrence and distribution of this fungus both in the Lake District and elsewhere are given in Tables VIII and IX.

TABLE VIII

Occurrence of Z. parvum *on* Sphaerocystis Schroeteri *Chod. in the Lake District from January 1949 to June 1950*

Lake.	1949.	1950.
Blelham Tarn	May 2–July 25 Sept. 12	—
Esthwaite Water	June 27–Aug. 27	May 30–June 5
Windermere: South Basin	Apr. 6–Oct. 3	June 5
Windermere: North Basin	Apr. 19–Oct. 3	May 24–June 5
Loweswater	May 18–Aug. 10	June 14
Hawes Water	July 30–Oct. 10	—
Wast Water	Sept. 7–Oct. 3	—
Ullswater, West	Aug. 17	—
Ullswater, East	Aug. 17–Sept. 14	—
Brothers Water	July 20	—

TABLE IX

Records of Z. parvum *(excluding English Lake District) from isolated preserved samples*

The host in all cases is *Sphaerocystis* except * where it is *Kirchneriella* sp.

Country.	Lake.	Actual date of collection.	
England	Swithland Reservoir	May 8	1938
		May 23	1946
		Apr. 26	1950
	*Newton Mere, Cheshire	Aug.	1922
Wales	*Rhosneigr Lake	June 22	1936
Ireland	Loch Currane	The West Collection, Birmingham, tube undated	
France	Lac Léman	Jan. 25	1950
Denmark	Viborg Nørresø	July 18	1949
Switzerland	Pfaffikersee	June 24	1948
Italy	Lago Misurina	Aug. 8	1910
Canada	Great Slave Lake	Aug. 21	1946

SUMMARY

Three chytrids are described from the English Lake District, namely, *Rhizosiphon crassum* Scherffel, *Rhizophidium fulgens* n.sp., *R. hyalobryonis* n.sp. After examination of material of *Phlyctidium anabaenae* Rodhe et Skuja from Sweden further details of the life-history are available and as a result the fungus is transferred to the genus *Rhizosiphon.* The so-called *sekundär zoosporangien* or ?*Dauerzellen* are considered to be sporangia of a hyperparasite. Further records from the Lake District and other localities are given for the following species: *Amphicypellus elegans*, *Chytriomyces tabellariae*, *Dangeardia mammillata*, *Zygorhizidium parvum*, *Z. melosirae*, and *Rhizidium Windermerense.*

My thanks are due to Drs. W. Rodhe and H. Skuja, Uppsala, Sweden; Dr. V. Tonolli, Pallanza, Italy; Mr. T. Christensen, Denmark; Dr. B. Dussart, Thonon, France; Professor J. Rawson, Canada; Mr. T. Griffin, Leicester

Water Company; Mr. N. Woodhead and Mr. R. D. Tweed, University College of North Wales, Bangor; Professor T. G. Tutin, University College, Leicester; Professor W. Stiles, The University, Birmingham; Lt.-Col. E. F. W. Mackenzie, Metropolitan Water Board, London, and Mr. J. Young, Hawes Water, for kindly sending samples of plankton or allowing me to work through their collections for fungi which are recorded in this paper. Especial thanks are due to Mr. G. Thompson of the Freshwater Biological Association for his excellent organization of the Lake District sampling and to the members of the laboratory staff who have helped to collect plankton samples. I am indebted to Dr. C. H. Mortimer for the German translations and to the Central Research Fund of the University of London for a grant enabling me to purchase a microscope which was used in this investigation. Lastly I am most grateful to Professor C. T. Ingold for his helpful criticism of the manuscript.

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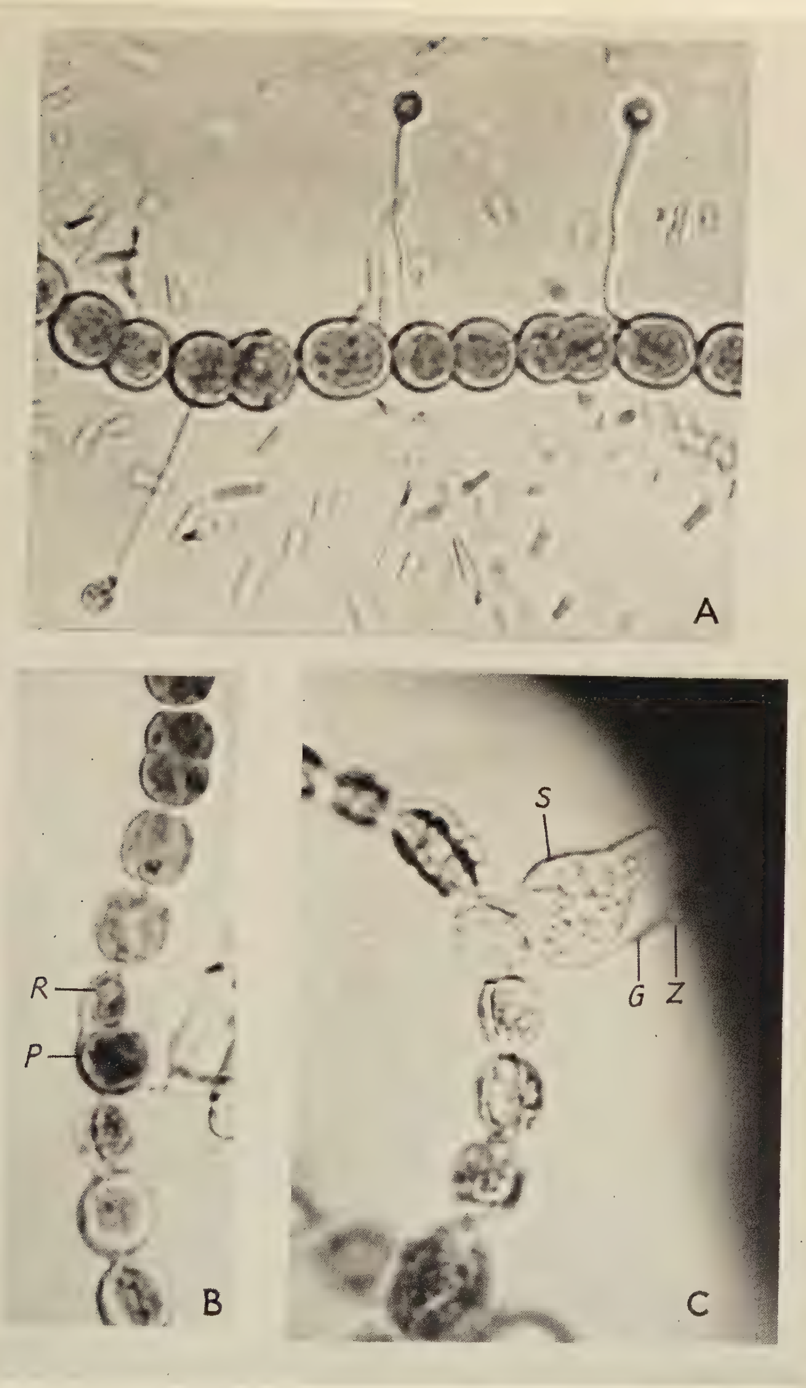

Rhizosiphon crassum on *Anabaena* sp. from Loweswater.

A. Three zoospores each with a fine germ tube to a host cell. × 1540. B. Prosporangium (*P*) with lateral tubular rhizoidal system (*R*). × 1520. C. Part of an *Anabaena* filament mounted in indian ink. Mature sporangium (*S*), empty zoospore cyst (*Z*), and germ tube (*G*). × 1540.

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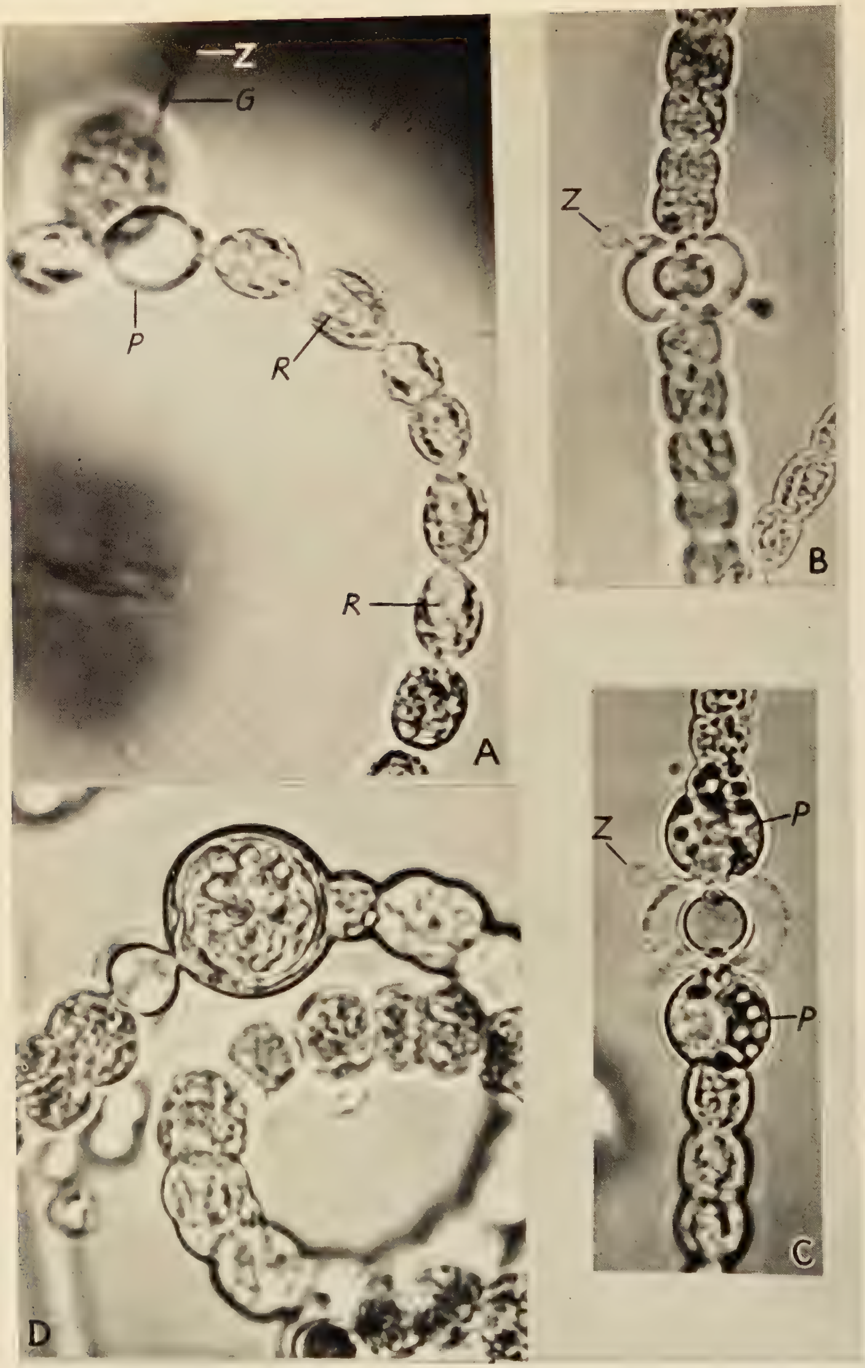

A. *Rhizosiphon crassum.* Part of an *Anabaena* filament mounted in indian ink, showing empty lateral rhizoid passing through six host cells (*R*), the double nature of the wall of the cell which contains the empty prosporangium (*P*), empty encysted zoospore case (*Z*), and its germ-tube (*G*); the sporangium is out of focus. × 1600.
B-D. *Rhizosiphon anabaenae.* B. Zoospore (*Z*) on heterocyst with short tube to neighbouring vegetative cell. × 1300. C. Swollen vegetative cells containing prosporangia (*P*); one empty zoospore cyst (*Z*) can be seen. × 1300. D. Resting spore. × 1300.

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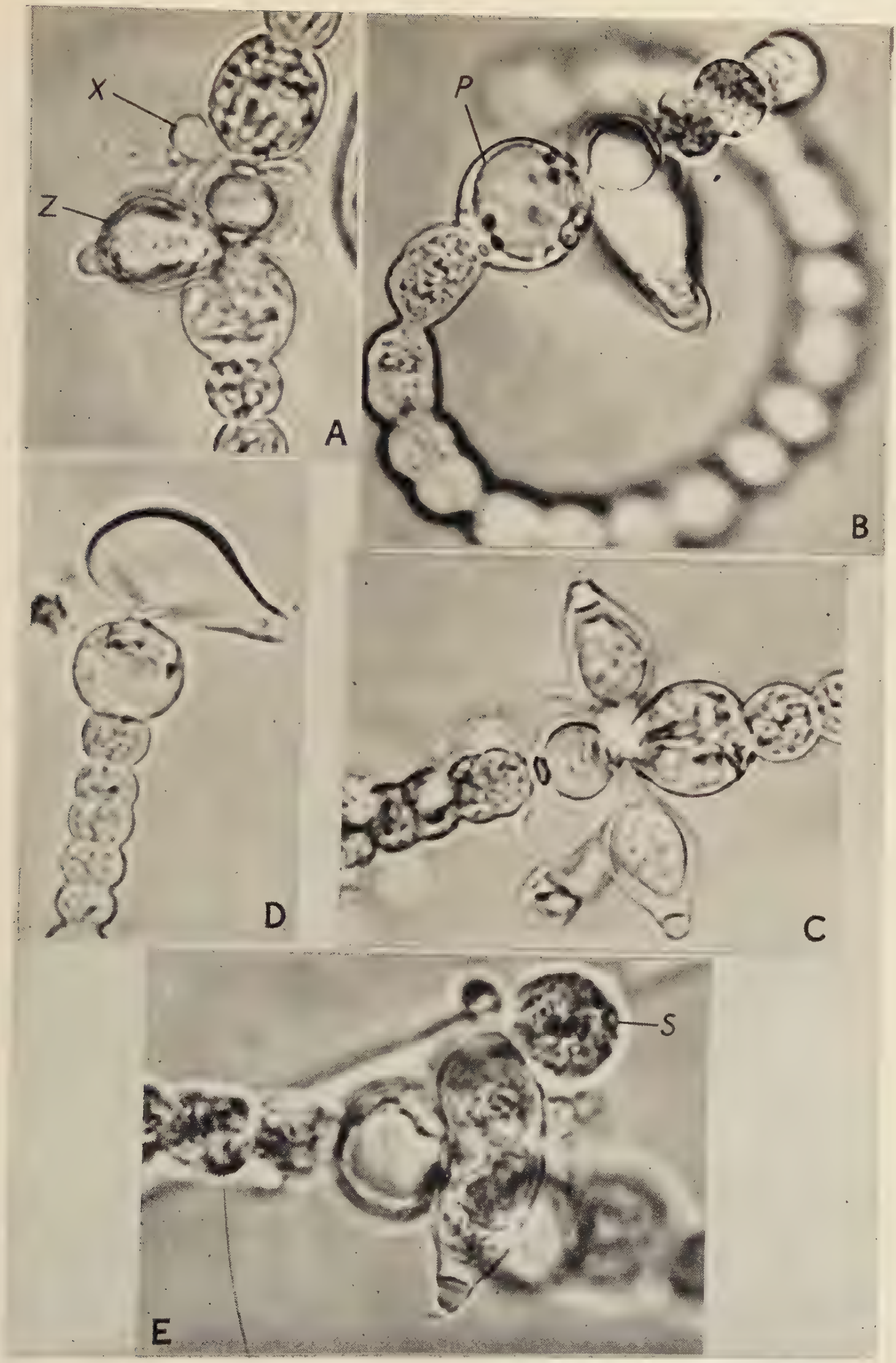

Rhizosiphon anabaenae Rodhe et Skuja nov. comb.

A. At (*X*) bud-like protuberance, i.e. early stage in the development of a sporangium. (*Z*) a sporangium out of focus. Note swollen host cells on either side of heterocyst. × 1330 B. Empty spherical prosporangium (*P*); the sack-shaped sporangium is out of focus. × 1270. C. Two, small, pear-shaped sporangia; both have a well-developed mucilaginous apex. × 1270. D. An empty sporangium. × 1270. E. Spherical sporangium (*S*) of fungus infecting *R. anabaenae*. × 1290.

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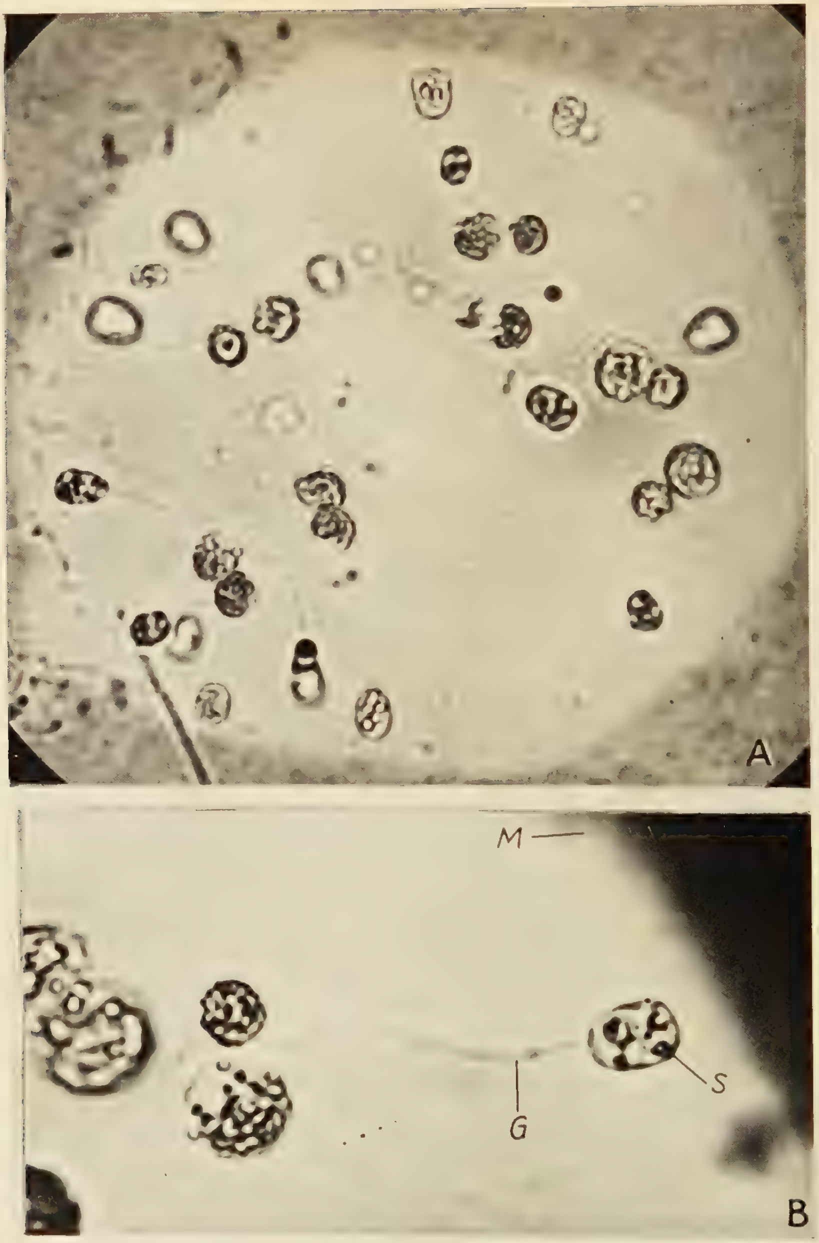

A. A colony of *Gemellicystis neglecta* bearing numerous sporangia of *Rhizophidium fulgens* n.sp. × 700. B. A sporangium of *Rhizophidium fulgens* (*S*), its unthickened germ tube (*G*) out of focus; (*M*) edge of mucilage envelope of host colony. × 1900.

H. M. CANTER

A Quantitative Study of Shoot Development in *Vicia faba*

II. The Elongation of Internode and Petiole

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With eleven Figures in the Text

ABSTRACT

The elongation of the internodes and petioles of *Vicia faba* has been studied in the plumules of normal seedlings, in seedlings from which part or all of the cotyledonary reserve has been removed, and in axillary shoots developed on seedlings of various ages.

The curves obtained by plotting the lengths of the internodes against their plastochrone ages reveal constant differences in behaviour between plumules and axillary branches. As the growth of any shoot progresses a greater contribution to the total elongation rate comes to be made by the internodes farther removed from the apex.

The length of each internode depends throughout its development on its position in the stem as well as on the length of the internode below. The extent to which the elongation of an internode lags behind that of the one below increases upwards, a relationship similar to that previously shown to exist in respect of the vascularization of the internodes.

In plumules the length of petiole corresponding to any given length of internode undergoes a decrease followed by an increase as the plumule is traversed from base to apex, a relationship exactly opposite to that previously demonstrated between the vascularization of a leaf-trace bundle and that of the internode in which it runs.

The effects of age and of removal of cotyledonary reserves are described, and it is concluded that it is unlikely that the characteristic differences in internode length within the limits of a single shoot are wholly due to changes in the availabilty of reserve substances.

INTRODUCTION

IN the first paper of this series (Dormer, 1950), which dealt with the development of the xylem in the plumule, complex relationships were shown to exist between the development of a leaf-trace bundle and that of other bundles in the same transverse section of the stem, and between the vascularization of one internode and that of the one below. The accumulation of large numbers of observations by anatomical investigation is such a slow process that it was thought desirable to extend the scope of the work by using other quantities which could be more rapidly measured. The present paper

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is based entirely on measurements of the lengths of growing petioles and internodes. These quantities are extremely easy to determine, so that large numbers of observations can quickly be obtained. Moreover, very few measurements of this kind have previously been published. It is true that a number of workers have discussed the problem of internode length, but they have been concerned almost exclusively with the final length of the internode after growth in length has ceased (e.g. Hales, 1727; Groom, 1909; Klebs, 1926; de Haan and Gorter, 1936). Investigations of stem elongation have for the most part been concerned only with the growth of the stem as a whole, and the analysis of the growth curves obtained has been carried on without reference to the natural subdivision of the stem into internodes.

Material and Methods

(*a*) *Cultivation and experimental treatments*

Seeds of broad bean ('Claudia') were sown individually in ordinary potting soil in 3-in. pots in a heated greenhouse. Apart from watering and the prompt removal of unwanted lateral shoots the only attention given was that involved in carrying out the operative treatments described below.

The plants to be described in this paper fall into eight classes, which it will be convenient to denote by the following system of symbols:

NP—Intact seedlings, giving plumules of normal structure.

$\frac{1}{2}$2cot*BG*—The seed is cut in half (as accurately as can be judged by eye) after it has had time to absorb water, but before the testa has ruptured. In this way the distal half of each cotyledon is removed, together with half the testa, and the plant develops from the earliest stages of germination with only about half the normal quantity of cotyledonary reserve substances.

1cot*AG*—The seed is sown intact, and germinates in the ordinary way until the plumule is just showing above the soil. The cotyledons are then laid bare, and one of them is severed at its extreme base and removed, care being taken not to disturb the root system. The earth is then replaced and growth allowed to continue. In this way the seedling is deprived of half its remaining cotyledonary reserves at a time when the plumule is partly above ground but before its photosynthetic activity can have made any appreciable contribution to the nutrition of the plant.

2cot(4)—The seed is sown intact and the plant allowed to develop without disturbance until it has reached the age of four plastochrones (regarding the convention for measurement of plastochrone age, see Dormer, 1950, p. 424). Both cotyledons are then removed. By this method the plant is deprived of all remaining cotyledonary reserves as soon as the first foliage leaf (leaf 3 of the plumule) has unfolded. Microscopic examination of the cotyledons removed at this stage shows that they still contain large quantities of starch.

In the plants so far described the shoot studied was the plumule, any lateral shoots which might develop being cut off as soon as possible. In the remaining categories the upper part of the plumule was removed, and measurements were made on an axillary branch which was thereby induced to develop. In every case the plumule was severed in its second internode, and the shoot which was allowed to develop was the secondary bud of the first scale leaf (leaf 1 of the plumule). It was necessary to use the secondary bud because in some of the older plants the primary bud had already grown out spontaneously and been removed in the course of routine pruning. If it had not already been destroyed before the decapitation of the plant it was removed as soon afterwards as possible. These operations were performed on plants of various ages, so that we have to distinguish the four classes (3)*CD*, (4)*CD*, (5)*CD*, (6)*CD*, the number in brackets indicating the plastochrone age of the plant at the time of decapitation.

Under the conditions ruling during the course of this investigation the mortality due to the operations just described was negligible, and growth continued without serious interruption, though at a reduced rate. Certain other treatments, the nature of which will be readily understood from the symbols 2cot*AG*, (7)*CD*, (8)*CD*, (9)*CD*, (10)*CD*, resulted in mortality rates often in excess of 50 per cent., and the surviving plants in many instances showed little or no growth for long periods. The observations on these plants are not described in the present paper, since, being based on a selection of plants (i.e. on those that survived), they cannot legitimately be compared with the results of experiments where all the plants survived.

(*b*) *Measurements*

The lengths of internodes and petioles were measured to the nearest millimetre by means of a ruler and a pair of dividers. It is necessary in such work to define as precisely as possible the criteria by which the position of a node is to be determined. In this species it is most convenient to take the position of the node as the point where the lateral leaf-trace branches off from the cortical bundle and turns out towards the leaf. This point can be located with sufficient accuracy from an examination of the outer surface of the stem, the course of the outgoing leaf-trace being marked by a distinct swelling. The length of an internode has thus been measured along one of the lateral ridges of the stem, from the point of departure of one lateral trace to that of the next. The length of a petiole has been measured from the point of departure of a lateral leaf-trace to the angle where the basiscopic margin of the leaflet on the same side meets the upper edge of the petiole. The length of the *n*th internode will be denoted by I_n and that of the *n*th petiole by P_n, both being expressed in millimetres throughout.

In this investigation, as in the previous study of xylem differentiation, attention has been directed mainly to the parts of the plant which bear bifoliolate leaves. In the case of plumules, observations extend from the third internode to the one below that which bears the first leaf having more than two leaflets. In axillary branches the first leaf is often reduced to a scale, and then affords no measure of petiole length, though in such cases the internode has been allowed to contribute to the records of I_1. In axillary shoots, as in plumules, the observations cease at the uppermost bifoliolate leaf.

Readers will be aware that in *Vicia faba*, as in other members of the Vicieae, the shoot is dorsiventral, the lateral leaflets on one side of the leaf being nearer to the base of the petiole than those on the other side. This asymmetry, however, rarely affects the bifoliolate leaves of the seedling, so that it has not been necessary to distinguish between the two sides of the leaf in measuring the length of the petiole. A little difficulty arises in the measurement of the first internode of the axillary branch. I_1 has been measured from the point of departure of a lateral trace of the axillant leaf to that of a lateral trace of the first leaf of the branch, but the resulting figures are certainly less accurate than the measurements of other internodes.

In taking the measurements the routine has been to determine the plastochrone age of the plant and to record separately the length of each internode and petiole. No record was kept of the date of the measurements, since it was not desired to make any estimates of absolute growth rate. Nor was any attempt made to follow the history of a particular individual through successive measurings. Care was taken, however, that a given plant, once measured, was not measured again for some days; in particular, precautions were taken to see that the same internode was not measured repeatedly after its elongation had ceased. The data on which this paper is based thus consist of large numbers of simultaneous determinations of plastochrone age, internode length, and petiole length.

(*c*) *Precautions against error*

Apart from mechanical errors of measurement, the principal factors likely to lead to false conclusions in work of this kind appear to be the following:

i. *Individual variability.* This can only be dealt with by distributing the plants randomly among the various treatments and taking large numbers of observations. Any attempt at a formal statistical analysis would be of doubtful value, in view of the impossibility of achieving a uniform distribution of the other sources of error listed below.

ii. *Effects of position on the greenhouse staging.* It is impossible to avoid variations in the number of plants on the staging during the course of an experiment, so that a statistically impeccable arrangement of the pots is unattainable. A consistent effort to minimize position effects has nevertheless been made, the plants subjected to any one treatment being broken up into small groups and these groups spread as evenly as possible among the whole mass of growing material. In order to reduce the effect of differences in spacing, the pots were packed together as closely as possible, the necessary adjustments being made when plants were added or taken away.

iii. *Effects of non-simultaneity of growth.* The plants exhibit considerable differences in absolute growth-rate, partly inherent, partly owing to the experimental treatments. It follows that two groups of plants can never be grown under identical conditions, since the group with the lower growth-rate must experience environmental conditions which the members of the other group will escape by completing their allotted span of growth in a shorter time. Short of rendering cultural conditions absolutely constant there is no way of eliminating such errors. An attempt to minimize them has been made by ensuring that there were, growing on the staging at any given time, representatives of all groups and age-classes. Any change in conditions must then have affected some members of every group.

iv. *Effects of uneven early development.* Suppose that a batch of seed has been sown to provide a supply of *CD* plants. Obviously, some of the plants will reach the age of 6 plastochrones before others. If now the plants which are furthest advanced in their development are converted into (6)*CD* plants and some of the others into, say, (4)*CD* plants, the result of the experiment will be vitiated by the consideration that the two groups differ not only in the age at which decapitation was carried out but also in their inherent absolute growth-rates. It is probably impossible to eliminate errors of this kind unless one is prepared to waste a high proportion of the total number of seedlings grown. An effort has, however, been made to see that the plants subjected to any particular treatment were drawn as evenly as possible from both fast-growing and slow-growing individuals in each batch of seedlings.

THE GROWTH OF THE PLUMULE

The measurements of internode length can be studied by several methods. The most obvious procedure is to calculate the average value of I_n for every value of n and for every plastochrone age and to plot a series of graphs showing the increase of the length of each internode of a plant with increasing plastochrone age, as has been done for the normal plumule in Fig. 1 (continuous lines). As the growth of an internode is completed in a small number of plastochrones, and as the plastochrone time-scale does not admit of the estimation of fractions, the graphs are inevitably somewhat angular, but they are unmistakably sigmoid in form. Different internodes give graphs which are clearly of different shapes, but the differences are not of a kind which readily admits of brief verbal description, and for purposes of comparison it will be better to make use of the curves indicated in Fig. 1 by broken lines. Each of these curves is constructed by joining all points which refer to internodes of the same plastochrone age. It is obvious that the age of the third internode during the unfolding of the third leaf is the same as that of the fourth internode during the unfolding of the fourth leaf, while the age of the third internode during the unfolding of the fourth leaf is the same as that of the fourth internode during the unfolding of the fifth leaf, and so on. It follows that a consistent measure of the plastochrone age of a particular internode, as distinct from that of the plant as a whole, can be obtained by subtracting the serial number of the internode from the plastochrone age of the plant. In all this work each group of *CD* plants must be treated as having its own independent scale of plastochrones.

If, now, the values of *n* are marked off as abscissae, and the successive lengths of each internode marked off along the corresponding ordinate, a

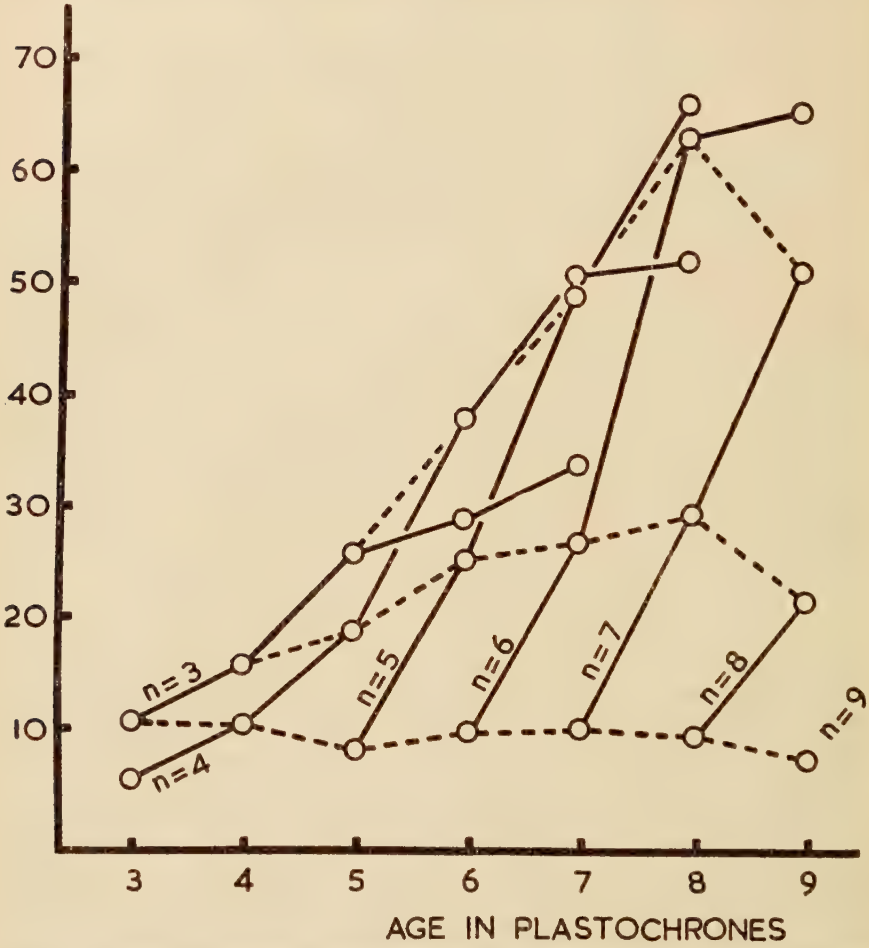

FIG. 1. Graphs of I_n against plastochrone age of the plant for normal plumules. Broken lines join points referring to internodes of the same age.

figure will be obtained in which the broken lines of Fig. 1 reappear in a slightly different arrangement, each one representing the variation of internode length with serial number when all the internodes are taken at equivalent stages of development. Each curve will show the condition of internodes one plastochrone older than those which generate the curve below, and all the curves may be regarded as successive approximations to the graph of final internode length against serial number, equivalent to the graphs which have been published by Groom (1909) and others.

In Figs. 2–4 the curves for the normal plumule are shown by broken lines; the corresponding curves for treated plants being shown by continuous lines. Fig. 2 gives the comparison for the 1cot*AG* plumule, Fig. 3 that for the $\frac{1}{2}$2cot*BG*, and Fig. 4 that for the 2cot(4). It will be seen that, as might be expected, the general effect of removing cotyledonary reserves is to reduce the lengths of the internodes, but that in places there are indications of a stimulating effect. In particular the $\frac{1}{2}$2cot*BG* plumule at first produces internodes which are considerably longer than they would have been if the seed had not been halved. In the higher internodes this treatment has a depressing effect on the process of elongation. In the 2cot(4) plumule there are only slight indications of a very transitory stimulation, followed by a period of pronounced depression. In the 1cot*AG* plumule, on the other hand, the elongation of the internodes is depressed from the first, but there is some indication of a slight stimulation in the later stages of development of the seventh and eighth internodes. The discussion of these results will be postponed until an account has been given of the petiole measurements, with which they are closely related.

The study of internode elongation by means of the graphs so far presented is handicapped by the imperfections of the plastochrone time-scale, particularly by the impossibility of estimating fractions. This difficulty can in some respects be overcome by plotting the length of an internode against that of the one below, the two measurements being taken simultaneously. In constructing these graphs of I_{n+1} against I_n it has been necessary to use the method of curve-smoothing introduced in the previous paper (Dormer, 1950). Owing to the greater variability of the length measurements as compared with the counts of xylem elements, the observations have here been grouped in twenties instead of in fives. The resulting graphs for the normal plumule are shown in Fig. 5. The curves show a steady fall towards the axis of I_n as the value of n increases, and should be compared with the curves already given for the vascularization of the internodes (Dormer, 1950, Fig. 3). The difference in development of high and low internodes is extremely conspicuous. When, for example, the third internode attains a length of 30 mm. the length of the fourth is nearly 40 mm., but when the eighth reaches a length of 30 mm. the length of the ninth is less than 10 mm. This change results in obvious differences in general appearance between the growing regions of seedlings of different ages.

The removal of cotyledons seems to have little or no effect on the form of curves of this kind, the graphs for the experimental plumules (not shown) coinciding very closely with those for the normal plumule.

There is yet another way in which the measurements of internode length may be examined. Consider the region of the stem which is actively elongating. It is clear from the most superficial inspection that several internodes are growing simultaneously, and the question therefore arises as to how the total elongation occurring during any short period of time is distributed among them. It is obviously desirable to ascertain whether or not this

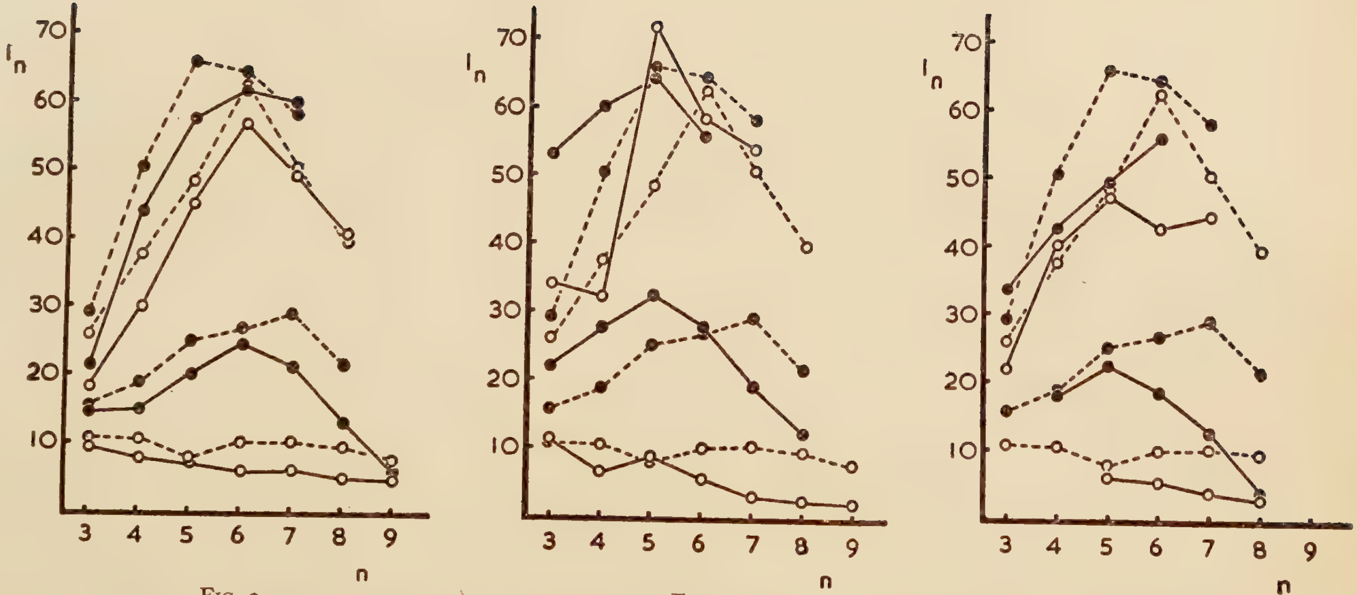

FIG. 2

FIG. 3

FIG. 4

FIGS. 2-4. Graphs of I_n against n. Broken lines refer throughout to normal plumules, continuous lines to plumules of treated plants Fig. 2, 1cotAG; Fig. 3, ½2cotBG; Fig. 4, 2cot(4).

distribution undergoes any characteristic change as the growth of the plant proceeds.

It is easy to obtain by subtraction the average elongation of a given internode during any given plastochrone, and to construct a table showing the mean elongation of each internode, in millimetres per plastochrone, for each plastochrone of the plant's age. It is convenient to arrange the figures so as to bring into the same column all those which refer to internodes of the same plastochrone age. That is to say, it is necessary to compare the elongation of the fourth internode during the plastochrone which elapses between the unfolding of leaf 6 and that of leaf 7 with the elongation of the fifth internode in the plastochrone which separates the unfolding of the seventh leaf from that of the eighth, and so on. As there are considerable variations in growth rate during the life of the shoot, it is convenient, in studying changes in the distribution of growth, to reduce the figures to percentages, the growth rate of the internode of zero age (i.e. the one associated with a leaf in the act of unfolding) being taken as 100 in all cases.

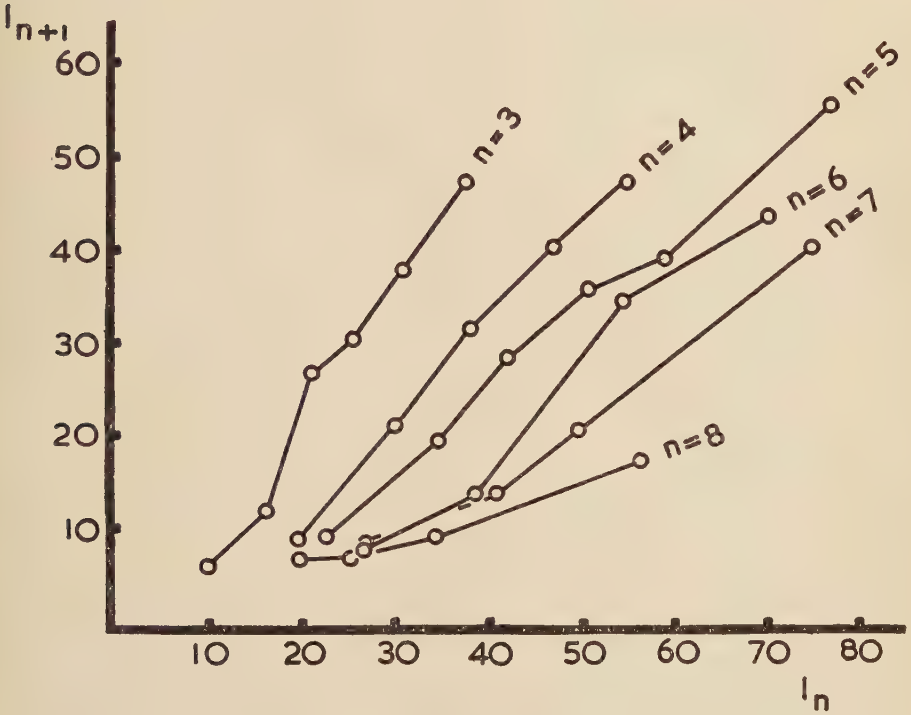

FIG. 5. Smoothed curves of I_{n+1} against I_n for normal plumules.

Table I gives the results of an investigation of the normal plumule, carried out in this way. As the age of the plant increases, the internodes farther removed from the apex make an increasingly important contribution to the total elongation of the shoot as a whole. It is also clear that after a very early stage of growth has been passed the greatest contribution to total elongation is made by the internode aged one plastochrone.

TABLE I

Age of plant	Age of internode				
	−1	0	1	2	3
3	92	100	—	—	—
4	—	100	115	—	—
5	—	100	111	19	—
6	—	100	139	74	28
7	—	100	188	88	7
8	—	100	180	15	—
9	—	100	259	106	—

The plumules of treated plants yield tables not essentially different from Table I. As a specimen, Table II gives the figures for the 1cot*AG* plumule. The tendency for measurable elongation to occur in a greater number of internodes during the sixth and seventh plastochrones is general, and coincides with a maximum of total elongation rates at about this time.

TABLE II

Age of plant	Age of internode				
	−1	0	1	2	3
3	86	100	—	—	—
4	37	100	73	—	—
5	—	100	113	21	—
6	—	100	131	77	52
7	—	100	223	86	—
8	—	100	333	58	—

All the methods which have been used in studying the elongation of the internode are equally applicable to that of the petiole. Owing, however, to the lesser extent and greater uniformity of petiole growth an analysis by these methods does not lead to any important new results. Apart from the graphs of P_{n+1} against P_n, plotted from the measurements of the normal plumule and given in Fig. 6 for comparison with the corresponding graphs of internode length (Fig. 5), the independent analysis of the petiole data is not worth reproducing.

It is more profitable to study the elongation of the petiole by constructing graphs of P against I. Graphs of this kind have been drawn for every internode of each type of plant. All these graphs have the same general character, those for the 2cot(4) plumules being shown in Fig. 7, where both variables are plotted logarithmically. Although all these curves have been subjected to a smoothing process there is still a good deal of overlap due to sampling errors. In order to determine whether there are constant differences between the curves for different values of n, it has therefore been necessary to employ the following method.

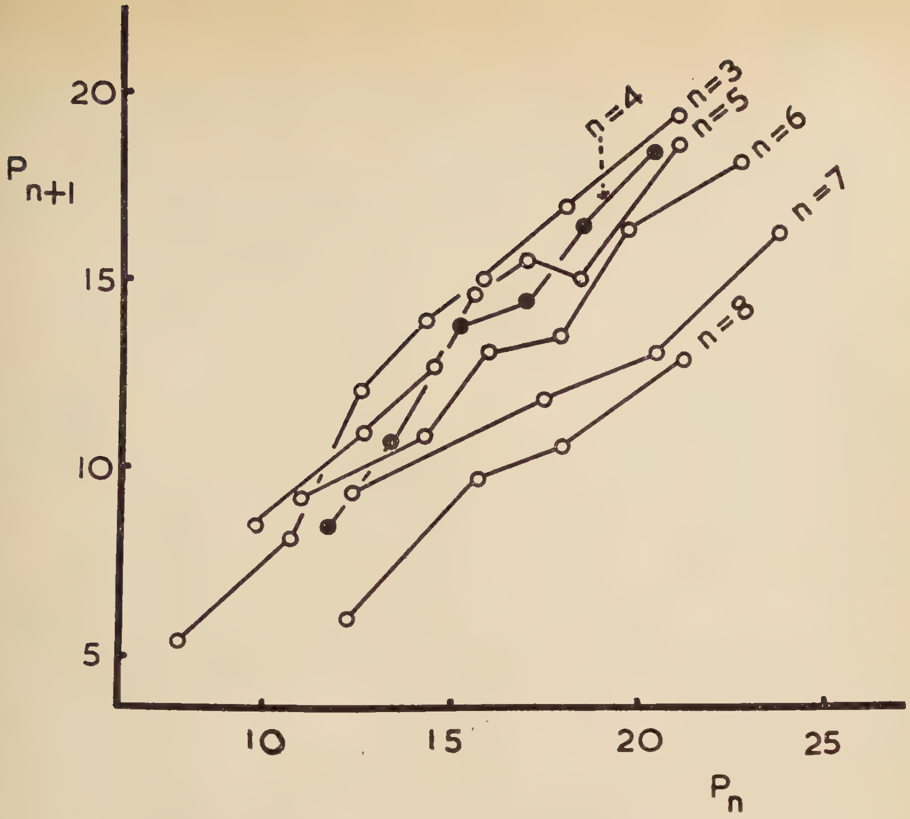

FIG. 6. Smoothed curves of P_{n+1} against P_n for normal plumules.

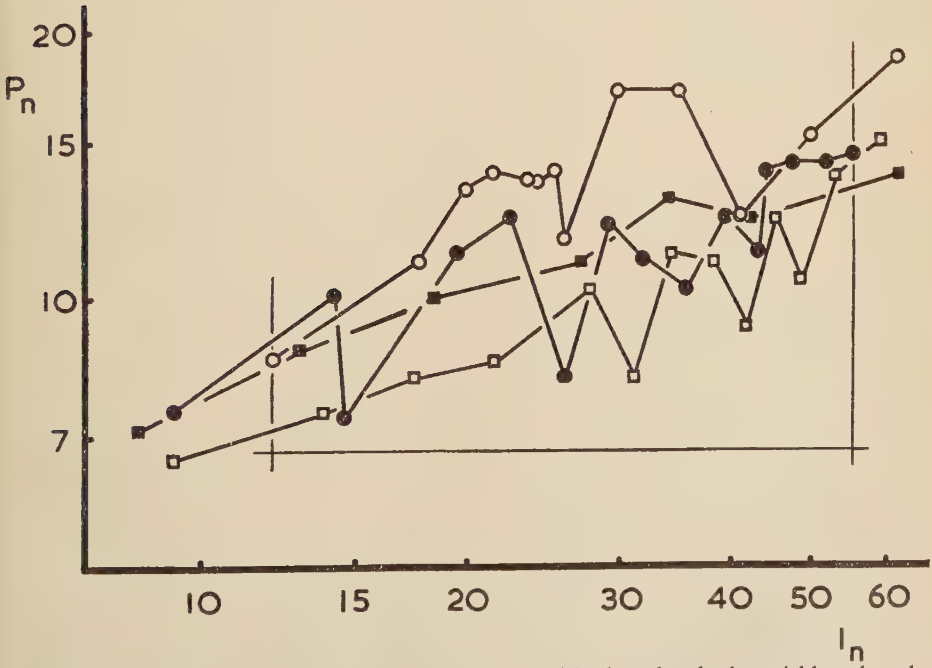

FIG. 7. Smoothed curves of P_n against I_n for 2cot(4) plumules, both variables plotted logarithmically. 3rd internode, white circles; 4th internode, black circles; 5th internode, white squares; 6th internode, black squares.

On each set of graphs two ordinates are drawn so as to cut off that portion of the total range of I over which values of P are available for all internodes. These two ordinates are then connected by a horizontal line close to the graphs. The area enclosed by the graph, the two ordinates, and the horizontal are then determined for each internode by the use of a planimeter. This area is an estimate in arbitrary units of the value of the expression

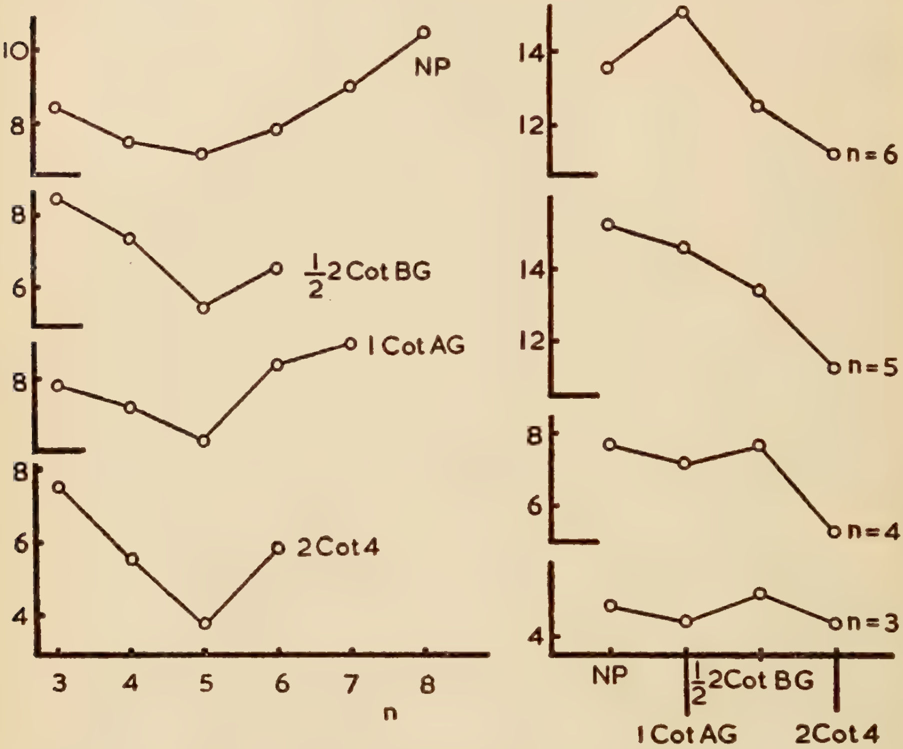

Fig. 8

Fig. 9

Figs. 8 and 9. Fig. 8, graphs of $\int \log P_n d(\log I_n) - C$ against n for comparing petiole lengths in successive internodes of the same plumule; Fig. 9, corresponding graphs to show the effects of experimental treatments on the relative length of petiole for each value of n.

$$\int \log P_n d(\log I_n) - C,$$

where C is an arbitrary constant and the limits of the integral are fixed by the overlap of the data.

The results of this work appear in Fig. 8, where the areas obtained in this way are plotted against n for each class of plumule. It is evident that in all cases the length of petiole for a given length of internode undergoes a decrease, followed by an increase, as n increases, the fifth petiole being the shortest in relation to the length of the corresponding internode.

These curves are not capable of revealing the effects of the experimental treatments, since the limits of the integrals and the values of the arbitrary constant C are different for each class of plants. In order to determine the effect of removing the cotyledons it is necessary to replot the graphs of P against I, collecting on one sheet all the curves which refer to the same value of n, and repeating on each sheet the construction of two ordinates and a horizontal. The areas obtained in this way are set out in Fig. 9, the lines in this figure being in effect graphs of $\int \log Pd(\log I)$ against the severity of experimental treatment, since the various treatments have been placed in such an order that the quantity of reserve substances removed increases from left to right.

For every value of n the length of petiole for a given length of internode is less for the 2cot(4) plumule than for any other, but the results of the other treatments are more complicated. The 1cot*AG* treatment results in a decrease in the relative lengths of petioles 3, 4, and 5, but an increase in the relative length of petiole 6. The ½2cot*BG* treatment, on the contrary, increases the relative length of petiole 3, but produces a relative shortening of the petioles of higher leaves.

A comparison of these results with the observations regarding internode length will show that there is a close parallel between the two sets of phenomena. The 2cot(4) treatment, which leads to a general shortening of the internodes, also produces a reduction in the relative lengths of all the petioles. The 1cot*AG* plumule at first produces abnormally short internodes, bearing petioles which are abnormally short even for internodes of the reduced length, while the upper internodes ultimately exceed the normal length and bear petioles which are abnormally long even for the increased length of internode. The ½2cot*BG* plumule acts in a precisely opposite manner, the lower internodes being lengthened, with excessively long petioles, the upper ones short with relatively short petioles. These relationships may be summarized in the statement that any effect which the removal of the cotyledons may have on the elongation of the internode tends to be accompanied by a similar but more pronounced effect on the growth of the petiole.

THE GROWTH OF THE AXILLARY BRANCH

The analysis of the measurements of the *CD* plants has proceeded on much the same lines as that of the plumule data, and can be described quite briefly.

In Fig. 10 are shown graphs of I_n against n, comparable with those given for plumules in Figs. 2–4, and referring to (3)*CD* plants (broken lines) and (6)*CD* plants (continuous lines). In Fig. 11 the corresponding graphs for (4)*CD* plants (broken lines) and (5)*CD* plants (continuous lines) are similarly compared.

All four sets of curves differ in a characteristic way from those obtained from measurements on plumules, owing to the precocious elongation of the first internode in all the axillary shoots.

The comparison of the (6)*CD* shoot with the (3)*CD* shows that apart from some indication of a slight and transitory stimulation of the growth of the first internode the effect of prolonging the period of ageing of the plant prior to decapitation is merely a general reduction of all internode lengths, a reduction which applies at every stage of development. The (4)*CD* and (5)*CD* shoots show a similar relationship with the (3)*CD*, but when they are compared with one another, as in Fig. 11, it becomes apparent that the (5)*CD* plant, during a considerable part of its early development, produces internodes which are actually longer than those of a (4)*CD* plant. The internodes of a (6)*CD* plant tend to be shorter than those of a (5)*CD*. As in the case of the plumules, these results run parallel with the relationships regarding petiole length.

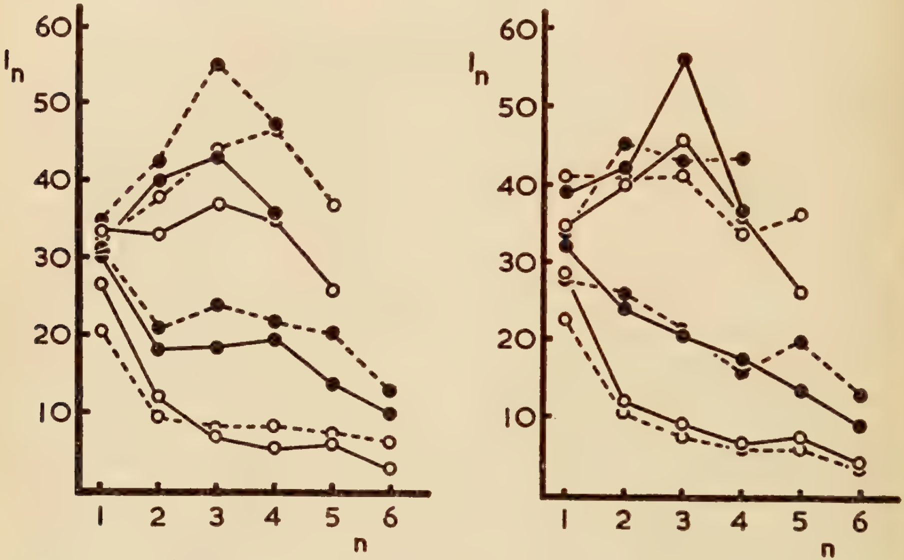

FIG. 10

FIG. 11

FIGS. 10 and 11. Graphs of I_n against n for axillary shoots. Fig. 10, (3)*CD* shoots (broken lines) and (6)*CD* shoots (continuous lines); Fig. 11, (4)*CD* shoots (broken lines) and (5)*CD* shoots (continuous lines).

Apart from a conspicuous flattening of the graph of I_2 against I_1, resulting from the precocious elongation of the first internode, the graphs of I_{n+1} against I_n for the axillary shoots are of the same general form as those derived from the plumules. If the fall in the gradient of successive curves be taken as a measure of physiological age, the axillary shoots must be regarded as showing some degree of rejuvenation as compared with the plumules from which they spring.

The distribution of growth among the internodes of the axillary shoot undergoes changes similar to those already seen in the plumule. The figures for (5)*CD* plants are given as a sample (Table III).

The early elongation of the first internode affects the shape of the graphs of *P* against *I*, making the curve for the first internode much steeper than the others, so that it cuts across them. Under these conditions it is hardly practi-

TABLE III

Age of plant	Age of internode				
	−1	0	1	2	3
1	209	100	—	—	—
2	29	100	23	—	—
3	—	100	150	40	—
4	—	100	222	14	—
5	—	100	284	171	65

cable to compare the first internode with others in respect of petiole length, and the first internode has therefore been omitted from the consideration of the change in petiole length along the length of the shoot. The values of $\int \log P_n d(\log I_n) - C$ appropriate for showing the effect of variations in n are given in Table IV, and indicate that the relative length of the petiole falls as n rises. There is no indication, within the range of the data, of any reversal of this tendency. The integrals required in considering the effect of ageing of the plant prior to decapitation are given in Table V. Although the frequent occurrence of a scale leaf at the first node introduces some uncertainty as to the comparative value of the observations, the first internode has been included in this analysis. The figures in the table agree in showing that the relative length of the petiole is less for (4)*CD* plants than for (3)*CD*, greater for (5)*CD* than for (4)*CD*, and less for (6)*CD* than for (5)*CD*. With only two exceptions among a total of twelve comparisons, the relative length of the petioles of (4)*CD*, (5)*CD*, and (6)*CD* plants is less than that of (3)*CD* plants.

TABLE IV

Plant	Internode			
	2	3	4	5
(3)*CD*	1470	1308	1302	1253
(4)*CD*	1096	1014	1035	886
(5)*CD*	1253	1261	1162	—
(6)*CD*	1444	1439	1262	—

TABLE V

Internode	Plant			
	(3)*CD*	(4)*CD*	(5)*CD*	(6)*CD*
1	284	241	247	157
2	1294	1185	1192	1236
3	1441	1384	1505	1490
4	1436	1402	1429	1339

DISCUSSION

Taken in conjunction with the results of the previous investigation of xylem development (Dormer, 1950), the facts now available regarding the lengths of the internodes and petioles of the normal plumule suggest that there must be an intimate physiological relationship between the elongation of an organ and its vascularization. In the case of the internode, this relationship manifests itself in the similarity between the graphs of I_{n+1} against I_n (Fig. 5) and the corresponding graphs for xylem development (Dormer, 1950, Fig. 3). In order to account for this similarity it seems necessary to suppose that an internode differs from one above it in having a higher value of some physiological factor, a difference which finds expression at first in a higher relative elongation-rate, and afterwards, when elongation has ceased, in a greater rate of vascularization.

In the case of the petiole the connexion between elongation and vascular development appears in quite a different form. It has been shown (Dormer, 1950, Figs. 4–6) that the degree of vascular differentiation in a leaf-trace bundle for a given degree of vascularization of the internode in which it runs is greater in the case of leaves 4 and 5 than for the leaves above or below. As shown in Fig. 8 of the present paper, the relative elongation of the petiole is at a minimum for the fifth leaf. That is to say, there is a negative correlation between the length of petiole for a given length of internode and the degree of vascular differentiation of the leaf-trace for a given degree of vascularization of the internode. The simplest hypothesis capable of accounting for this relationship seems to be that some factor is required both in elongation and in xylem differentiation, and that if more is utilized in one process there must be less available for the other.

A survey of all the available graphs of P against I leaves no doubt that the relationship between these quantities is not allometric. Over the range considered, the curves, when plotted logarithmically, show a steady decrease in gradient as I increases. The analogous curves for vascular differentiation appear to be of a flat sigmoid form, and it is quite possible that the graphs derived from length measurements are merely the upper portions of curves of the same kind.

It thus appears likely that the relations between elongation and vascular differentiation are, mathematically, extremely simple. If this be the case it is to be expected that similar relationships will be found in the axillary branch, the vascular development of which has not yet been examined.

With regard to the effects of experimental treatments, it is to be noted in the first place that the changes brought about by ageing the plant prior to the induction of an axillary branch are not essentially different from those produced in the plumule by removing cotyledonary reserves. Either operation may, according to the exact nature of the treatment, bring about an increase or a decrease in elongation of petiole and internode, and both tend to produce a more marked effect on the petiole than on the internode. In assessing these results it should be borne in mind that the integrals used in comparing the relative lengths of petioles are, in effect, averages, each being based on a number of observations which is always large and sometimes exceeds 100. These integrals certainly afford a more reliable indication of the effect of an experimental treatment than do the graphs of internode length, where each point is based on a smaller number of observations.

The changes in length of internode and petiole brought about by ageing or by removing cotyledons differ from those that normally occur during the life of an individual shoot, in that the greater length of the middle internodes of any plumule is accompanied by a relative shortening of the petioles. So far as the evidence goes, it seems unlikely that the characteristic differences in internode length within the limits of a single shoot are due to changes in the availability of reserve substances, since one would expect such changes to produce relatively longer petioles where the internodes were longer. On the other hand, there appears to be no morphological reason why the changes produced by ageing the plant prior to the development of an axillary shoot should not be attributed to partial starvation resulting from the preliminary depletion of the cotyledonary reserves.

The growth-patterns of the plumule and of the axillary shoot seem to differ irreconcilably. From the standpoint of the present investigation no internode of the one can be regarded as equivalent to any internode of the other. Nor can it be claimed that any of the treatments so far applied has in any way reduced the differences between the two types of development.

This work was carried out during the tenure by one of us (R. L. P.) of a Ministry of Education Scholarship.

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The Endosperm in *Rothia trifoliata* Pers.

BY

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With eleven Figures in the Text

ABSTRACT

After fertilization the ovule in *Rothia trifoliata* shows the development of a large multinucleate endosperm, which extends by haustoria at the expense of the cells in the base of the ovule. Later, this endosperm is almost entirely replaced by a relatively massive embryo.

ROTHIA trifoliata Pers., belonging to the tribe Genistae of the Papilionaceae, is a much-branched annual found in dry waste places. In Mysore, South India, it flowers profusely between July and October. Floral material was fixed in Randolph's modification of Navaschin's fluid and stained in Heidenhain's iron-alum haematoxylin for the examination of the ovules.

The ovules are arranged on a marginal placenta and are anatropous at the beginning but later become amphitropous. They also assume a curvature on the micropylar side. The outer integument, which forms the micropyle, is two-layered at the sides but more massive at its apical end. The inner integument is also two-layered, but it is shorter and ends just near the top of the nucellus. The nucellus comprises about three layers of cells in addition to some crushed cells immediately outside the embryo sac. A normal egg apparatus is organized at the micropylar end, the polar nuclei fuse before fertilization, and the three antipodals degenerate soon after fertilization (Figs. 1, 2).

The primary endosperm nucleus which is situated in the middle of the embryo sac (Fig. 2) divides to form two daughter-nuclei (Fig. 3) of which the micropylar moves up in the neighbourhood of the fertilized egg. The two nuclei next divide to form four (Fig. 4) and then eight. These nuclei are at first distributed uniformly, but a little later two or three of them situated in the chalazal half of the embryo sac become slightly enlarged and come to lie in a dense mass of cytoplasm on the funicular side of the ovule (Fig. 5). Here the embryo sac forms a papillate outgrowth which pushes into the nucellus, destroying the cells in its way (Fig. 6). The nucellar cells surrounding the upper two-thirds of the embryo sac are considerably larger than those situated lower down and towards the funicle. The enlarged endosperm nuclei, to which reference has already been made, move into the lateral extension or diverticulum of the embryo sac which now flattens and extends on both sides, disorganizing the nucellar cells adjacent to it (Figs. 7, 10). In the meantime

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the fertilized egg has divided and given rise to a small embryo. At the two- or three-celled stage of the proembryo a number of endosperm nuclei, surrounded by denser cytoplasm, gather at this end and form a sort of a collar around

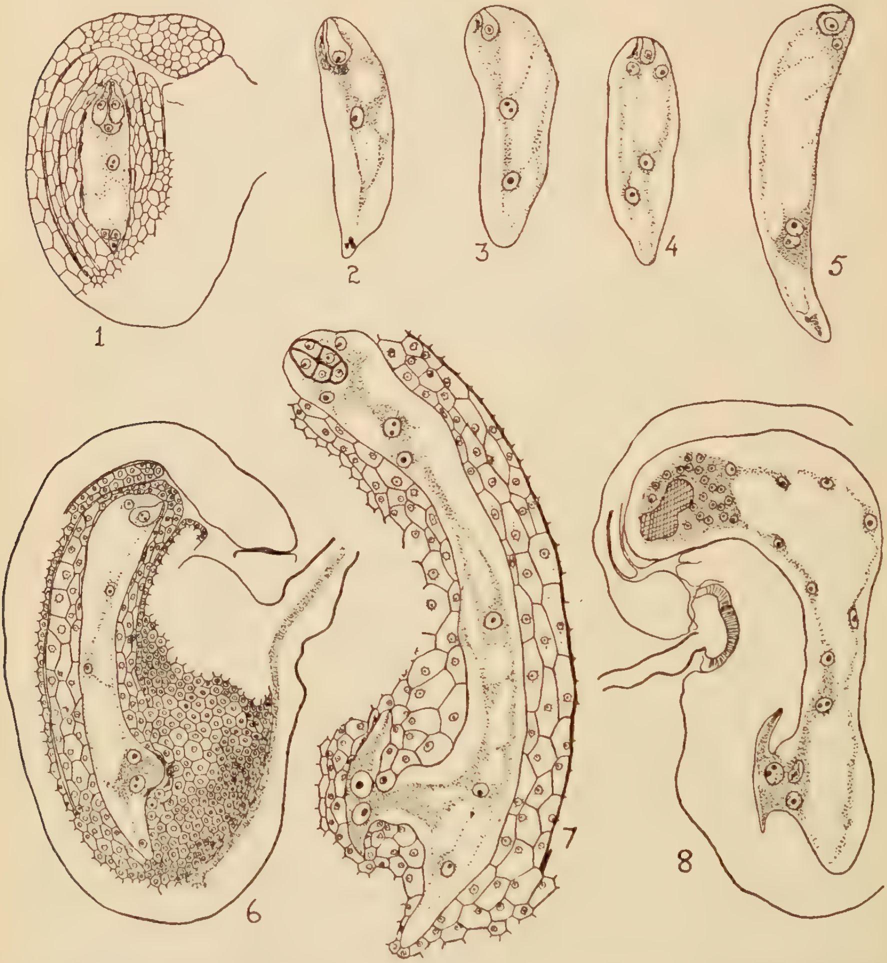

FIG. 1. Section of ovule showing a fully organized embryo sac. × 260. FIGS. 2–5. Formation of nuclear endosperm: note the two enlarged endosperm nuclei surrounded by dense cytoplasm. × 260. FIGS. 6, 7. Stages in organization of the diverticulum × 260. FIG. 8. Older stage × 110.

the embryo (Fig. 8). Wall formation in the endosperm takes place in the micropylar half of the embryo sac, while the chalazal half remains in the free nuclear condition. In later stages the diverticulum shows hypertrophied nuclei which assume curious shapes and appear to be in an advanced stage of disorganization (Figs. 9, 11). Most of the nucellar tissue in this region is also disorganized by now. The endosperm is gradually consumed and the fully developed embryo occupies almost the entire space in the embryo sac. The seed coat is very thin and is composed of only two layers of cells.

Various types of haustorial processes have been described in plants having a cellular endosperm, but they are not of common occurrence in plants having a nuclear endosperm. The condition described in *Rothia* may be compared to the 'Divertikel' reported in some members of the Caryophyllaceae (Rocèn, 1927). In *Rothia* the migration of nuclei into the diverticulum does not occur at such an early stage as in members of the Caryophyllaceae, nor is the diverticulum so long and tubular as in the latter. However, it forms a considerable vertical extension, enabling it to function to maximum advantage. Although a detailed study was made of the development of the embryo and endosperm in a number of plants belonging to the Papilionaceae, such an activity of the endosperm has not so far been observed in any other genus. Its occurrence in *Rothia* is probably related to the small amount of tissue at the base of the embryo sac, so that the only appreciable source of nutrition is the massive tissue on the funicular side.

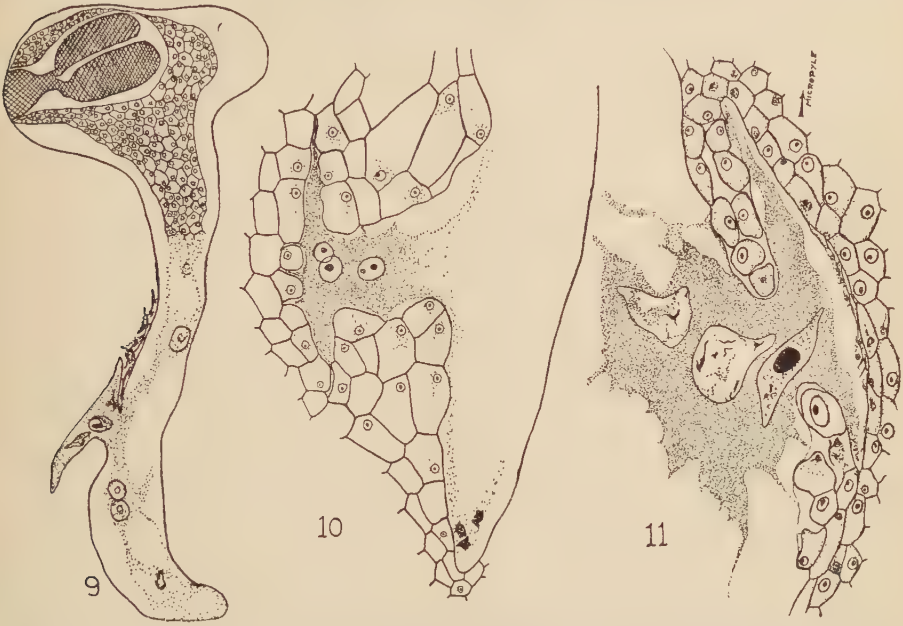

FIG. 9. L.S. of embryo sac showing cellular endosperm in the micropylar half and nuclear endosperm in chalazal half; note diverticulum with degenerating nuclei. ×110. FIG. 10. Magnified view of diverticulum. ×600. FIG. 11. Same, older stage. ×560.

The author expresses his gratitude to Professor P. Maheshwari of the Delhi University for the help given during this study.

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A Critical Study of the Auxin Theory of Growth Regulation in the Mesocotyl of *Avena sativa*

BY

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With three Figures in the Text

ABSTRACT

A method of growing *Avena* seedlings is described, which allows them to be handled individually in darkness.

Mesocotyls of seedlings from which the tip of the coleoptile is repeatedly removed are as long as those of control plants not so decapitated.

Mesocotyls of seedlings which are deseeded on the 3rd day of growth, followed by decapitation of the coleoptile tip on the 4th day, are, at 7 days old, as long as those of controls not so decapitated.

When deseeded plants are decapitated, regeneration of auxin production occurs at the tip of the coleoptile stump.

Where a reduction in the length of the mesocotyl results from decapitation, a wound reaction is probably concerned in addition to any auxin changes.

Removal of the coleoptilar node causes a sharp decrease in the final length of the mesocotyl.

Heating intact seedlings at 40° C. for 3 hours causes a reduction in the length of the mesocotyl but not of the coleoptile. The effect of heating is not reversed by subsequent treatment at low temperature, which instead appears to augment these effects.

When seedlings are exposed to the action of KCN, iodoacetate, or anaerobic conditions, and illuminated while so exposed, perception of light takes place, resulting in a reduction in the length of the mesocotyl.

Perception of light takes place in seedlings germinated at normal temperatures, but maintained at low temperature during illumination and also in seedlings grown for 6 weeks at 2° C. without any previous growth at normal temperature.

Light perception takes place in embryos excised from dry grain and grown on a culture medium.

No difference in free amino-acid content is apparent between dark grown and illuminated seedlings.

The effects of illumination survive a period of drying down and become apparent upon subsequent germination of the grain in darkness. The drying process itself causes an additional reduction in mesocotyl length.

It is concluded that auxin itself is not the primary reactant in the perception process, and that the growth of the mesocotyl is probably controlled by the coleoptilar node and plumular growing point, rather than by auxin diffusing downward from the tip of the coleoptile.

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I. INTRODUCTION

THE *Avena* seedling extensively used in hormone assays presents in its early stages of development an interesting series of co-ordinated growth phenomena.

When grown in complete darkness the mesocotyl, or first internode,[1] elongates rapidly from the 2nd to the 6th day when its growth virtually ceases, while the coleoptile (and the enclosed leaves) initially grows slowly, increasing its rate of elongation on the 4th day, and on the 8th day, when it has reached a final length approximately equal to that of the mesocotyl, its growth similarly ceases. The first leaf, however, continues to grow, breaks through the coleoptile, and elongates rapidly; root initials at the coleoptilar node, present in the early stages of development and carried up by the elongation of the mesocotyl, grow out diageotropically. These are called crown roots, and, as Goodwin (1941) has pointed out, they serve to anchor the young plant just below soil level.

Germination of the seedling under conditions of constant illumination results in the suppression of the mesocotyl, the elimination of the early period of slow growth by the coleoptile, and the earlier appearance of the crown roots. In general the coleoptile is, in these circumstances, shorter than when grown in darkness. Intermediary conditions of illumination produce mesocotyls having intermediate lengths, depending on the duration of the light period and the time at which exposure took place; the seedling increasing to maximum sensitivity some 2 days after soaking, thereafter decreasing as the 5th day is reached (Goodwin, loc. cit.).

The reasons for the suppression of the mesocotyl by light are not well understood. The explanation in terms of an auxin mechanism is critically examined in this paper. The results to be presented were obtained prior to the discovery of the effect of carbon dioxide in stimulating the extension growth of the mesocotyl (Mer and Richards, 1950), so that this factor is not here dealt with.

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[1] The term 'first internode' is now exclusively used in American literature, but 'mesocotyl' a shorter, well-understood term is used throughout this paper.

II. MATERIALS AND METHODS

Avena sativa var. 'Victory' was used throughout the investigation. The seeds were dehusked and planted, after soaking for 2 minutes in tap-water, in glass tubes $2\frac{1}{4}$ in. long and 6 mm. internal diameter, in each of which a slip of filter-paper $2\frac{1}{2}$ in. long by $\frac{1}{4}$ in. wide ($1\frac{1}{4}$ in. × $\frac{1}{4}$ in. when folded) had been placed. In practice it was found convenient not to fold the filter-paper exactly in half, but to leave an overlap which could then easily be located with the end of a pair of forceps, to part the two arms when inserting the seed at planting. The seed-holder was dipped into water to moisten the filter-paper, the seed was inserted, and then the damp filter-paper was pushed to the desired position, usually to bring the seed to $\frac{1}{4}$ in. from the top of the tube. It was found necessary to round off the sharp edges of the tubes in a flame prior to using them, as in preliminary work it was noted that otherwise many seedlings were inadvertently injured during subsequent handling, which took place in complete darkness.

These seed-holders were placed in groups of three in specimen tubes (2 in. by $\frac{3}{4}$ in.) partially filled with tap-water (pH 8) to reach the lower edge of the filter-paper; the seed was thus supplied with water by wick action (Raines, 1936; Kaiser and Albaum, 1939). The specimen tubes were grouped 19 at a time in small crystallizing dishes (10 cm. in diameter), the 57 seeds thus forming a set in which each seedling could be handled separately in darkness. The plants were grown under litre beakers, lined with damp blotting-paper to maintain a humid atmosphere, in cylindrical tins 6 in. in diameter and 9 in. tall fitted with lids to exclude light. These formed convenient portable dark chambers, and they could be carried from the incubator (at 22° C.) where the plants were grown to the dark room where decapitation or other treatment was carried out without danger of exposing the plants inside to light.

Further details of technique relevant to particular experiments are included in the appropriate place in the text.

III. EXPERIMENTAL

1. *Growth in darkness*

Fig. 1 shows the daily progress of growth of both coleoptile and mesocotyl; each point is the mean derived from a random sample of individuals which were then discarded. It will be seen that the mesocotyl grows rapidly at first, slowing down on the 5th day and virtually ceasing to grow on the 6th day. The coleoptile, however, grows much more slowly at first, but by the 8th day is approximately as long as the mesocotyl. It then similarly stops growing.[1]

---

[1] Laboratory air was unavoidably included in the tins, and later experiments carried out with air drawn from outside the laboratory was found to alter the growth of the coleoptile.

2. *Decapitation of the coleoptile tip*

Previously published results of decapitation experiments, which obviously involve the removal of the hormone-secreting tip of the coleoptile, are quoted in support of the auxin hypothesis of mesocotyl extension. This was stated by van Overbeek (1935) in the following terms: 'the growth of the mesocotyl is quite dependent on the amount of auxin left over by the coleoptile. If this amount is large, a large growth will result; if the amount is small, then little mesocotyl growth will result. This can be shown very nicely by decapitation experiments of normal plants. The amount of auxin produced by a new "physiological tip", if a tip of about 1 cm. is cut off, is considerably less than the amount produced by the original tip. Still this amount is sufficient for the coleoptile stump to grow as much as a comparable zone in the intact plant, but the growth of the mesocotyl is stopped completely.'

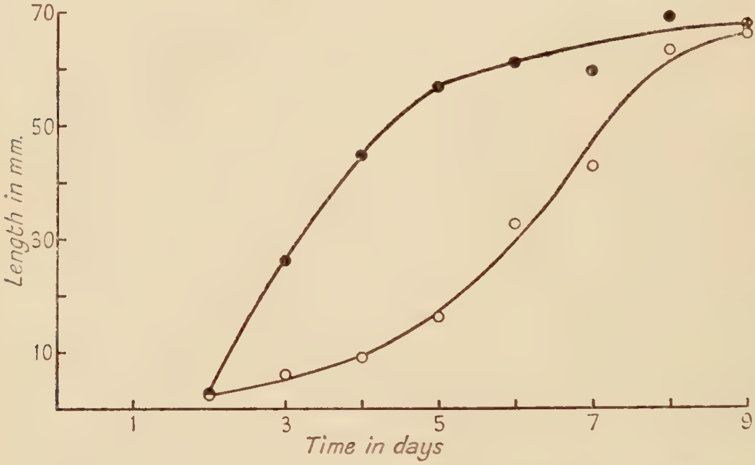

FIG. 1. Shows the progress of growth, at daily intervals, of the coleoptile (open circles) and the mesocotyl (full circles).

The results of Beyer (1927) and Inge and Loomis (1937) are relevant in this connexion. The former author decapitated his plants once only, and found a reduction in the length of the mesocotyl. He gave no indication of the time interval between decapitation and measurement, which needs to be known to determine the auxin-free period, and this makes the interpretation of his results somewhat difficult.

Inge and Loomis decapitated their plants (maize) 3 times in 24 hours ('at 8 to 12 hour intervals') and this was designed to circumvent the problem of regeneration. Dolk (1926) had shown, however, that regeneration took place in 2½ hours after decapitation, while Thimann and Bonner (1933) later reduced this interval to 1½ hours. It is evident, therefore, that 3 decapitations in 24 hours does not provide the solution to the problem as Inge and Loomis anticipated, and they admit that as the lengths of the treated mesocotyls were

40–42 per cent. of those of the controls, that an appreciable amount of regeneration must have taken place.

The literature on regeneration is somewhat confusing and contradictory. Skoog (1936/7) stated that Dolk demonstrated the suppression of regeneration by repeated decapitations (p. 313). This statement is incorrect, for Dolk showed that a second decapitation 2 hours after the first reduced the elongation to zero, with no implication about suppressing regeneration. His figure (1936, p. 514) showed quite clearly that elongation on a much reduced scale began again 60 minutes after the second decapitation. Without producing any further evidence to justify it, Skoog later reversed his initial statement (loc. cit., p. 320).

Van Overbeek (1937), on the other hand, credited van der Weij (1931) with showing that successive decapitations suppress regeneration; but this is not so, and van der Weij was careful to quote Dolk exactly: that immediately following the second decapitation, elongation was reduced to zero. He did, however, demonstrate that coleoptiles decapitated twice produced larger curvatures when a test block of agar containing growth substance was applied unilaterally than those decapitated once only and similarly treated, i.e. the former were more sensitive, owing to the greater depletion of residual auxin. Later in the paper referred to above, van Overbeek showed that regeneration took place in 90 minutes after a *second* decapitation, thus controverting his previous statement.

It is now a matter of common knowledge that in the standard *Avena* assay the agar block to be tested is put in position unilaterally, following the second decapitation of the coleoptile, and the ensuing curvature is photographed 90 minutes later. After this period has elapsed the curvature is gradually reduced owing to the straightening of the coleoptile, which process is ascribed to the regeneration of an auxin-producing tip on the side of the coleoptile remote from the test block. This explanation is, in consequence, based on the assumption of regeneration following the *second* decapitation, and there appears to be no experimental evidence for the initially quoted statements of Skoog and van Overbeek.

In view of this discussion on previous results, the present investigation was undertaken with the object of keeping the coleoptile auxin free for as long as possible, and finding the effect on the mesocotyl. Three-day-old plants, grown in darkness, were used, in which the mesocotyl was growing rapidly, and the coleoptile was approximately 5 mm. long; 5 decapitations of 1 mm. each were thus possible. It was therefore decided to lengthen the experimental period by decapitating at 2½-hour intervals instead of 1½ hours, to obtain as large a difference between the treated and control plants as was possible, for all were measured 2½ hours after the final decapitation. Control and experimental plants were grown together in the same tin in approximately equal numbers. As all subsequent operations were to be carried out in complete darkness, it was necessary to have some means of distinguishing by touch between control seedlings and those to be decapitated, and to ensure this the latter were grown as already described in 2$\frac{1}{4}$-in. seed-holders, while for the former, 2-in. long seed-holders were used, and these consequently did not protrude above the top of the specimen tubes. Selection of the longer seed-holders, therefore, automatically identified those for treatment. The plants were thus exposed for the same length of time to the temperature and atmospheric conditions prevailing in the dark room during decapitation, but at all other times they remained in the incubator at 22° C.

In all experiments an additional set of plants was grown for measurement just prior to decapitation for comparison with the final length of the mesocotyls of the treated plants. Decapitation was performed in darkness, as already mentioned, using a small implement consisting of a plate of brass $\frac{3}{4}$ in. × $\frac{3}{4}$ in. × $\frac{1}{32}$ in. thick, on to which two strips $\frac{3}{4}$ in. × $\frac{1}{4}$ in. × 1 mm. were soldered, to leave a median slot $\frac{1}{4}$ in. wide × 1 mm. deep (Fig. 2). A piece of razor-blade was cemented across the channel with sealing-wax, and the tip of the coleoptile was cut off by holding the plant, still in its seed-holder tube, vertically against the top of the channel, and then pushing the blade forward. To ensure that a slice had been removed, a small strip of brass was pushed along the slot, and so the piece cut off was recovered by touch. If none was found the operation was repeated.

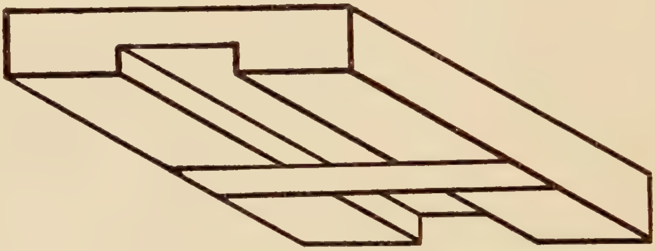

FIG. 2. The implement used for removing a standard length of the coleoptile tip in darkness (see text).

The results of two such experiments, involving 5 sets of plants, are recorded in Table I. Throughout this paper measurements are given in millimetres, and the number of replicates is quoted in brackets. The variability stated is the standard error.

TABLE I

The Effect of Repeated Decapitations of the Coleoptile on the Elongation of the Mesocotyl. (Experiments 2 and 3)

Experiment 2.	1st set.	2nd set.
Decapitated . . .	45·5±1·06 (11)	48·1±0·65 (20)
Controls (final length) .	47·5±1·05 (23)	49·7±0·89 (22)
Initial length of mesocotyl.	44·4±0·97 (22)	

Experiment 3.	1st set.	2nd set.	3rd set.
Decapitated . . .	38·4±1·06 (23)	40·3±0·8 (21)	40·5±1·09 (24)
Controls (final length) .	38·4±1·0 (27)	40·7±0·8 (33)	40·6±0·64 (27)
Initial length of mesocotyl.		34·6±1·57 (21)	

These figures indicate little difference between the final mesocotyl lengths of the control and decapitated plants, so that repeated removal of the coleoptile tip left mesocotyl growth unaffected. The results, therefore, are in conflict with van Overbeek's statement.

In expt. 2 the plants were decapitated 5 times at 2½-hour intervals, and measured 2½ hours after the final decapitation. It was found, however, that many plants were spoiled by cutting into the coleoptilar node at the final decapitation, so that in expt. 3 only 3 decapitations were given, measurements again being made 2½ hours after the last decapitation.

Some other method of maintaining the coleoptile free from auxin for a long period was therefore desirable, and a 'deseeding' technique seemed appropriate (Went, 1935). In describing this method, Skoog (1937) stated (p. 313), 'By deseeding, the source of auxin is removed; thus regeneration is prevented . . .'; while on p. 319, 'Thus, if the plants are decapitated about 15 hours after deseeding, subsequent regeneration of auxin is nearly completely prevented'. This technique seems to offer a simple method of maintaining an auxin-free coleoptile, and in the following experiments the plants were grown for 3 days as already described, when the endosperm was removed (in darkness) by taking each seed-holder in turn from the specimen tube, cautiously pulling the plant out until the seed could be felt, and detaching it between finger and thumb, and then replacing the seedling. The endosperm was quite soft at this time, and after a little practice could be removed without difficulty. The specimen tube, with the deseeded plants, was now placed in a spare crystallizing dish, which was gradually filled as treatment proceeded until all the plants which had germinated had been deseeded. Plants which were too small to handle and in consequence were left intact were discarded at the end of the experiment. A random sample of the 3-day-old plants was measured, and 24 hours later a sample of the deseeded plants was measured. Of the remainder, half were now decapitated, removing approximately 2 mm., and the others were left as controls for measurement on the 7th day. The results of two experiments are recorded in Table II.

Table II

The Effect of Deseeding and Decapitation on the Growth of the Mesocotyl (Means in mm. Number of Replicates in Brackets)

		Experiment 4.		Experiment 5.	
		Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
3-day-old seedlings . . .		16·0±2·8	5·2 (33)	17·8±0·86	5·3 (38)
Deseeded on the 3rd day	Measured on 4th day .	32·1±1·3	6·7 (15)	37·4±0·76	6·5 (37)
	Decapitated on 4th day and measured on 7th day . . .	44·9±1·3	— (23)	52·0±0·32	*10·8±0·53* (42)
	Controls, measured on 7th day . . .	46·7±1·2	15·4 (22)	50·1±0·32	17·1 (37)

The results again show no significant difference between the final mesocotyl lengths of the decapitated and control plants, and that growth has taken place after the removal of the coleoptile tip. If regeneration were in fact prevented by deseeding, then the average length of the coleoptile stumps (at 7 days) should be shorter than the control coleoptiles (at 4 days) by the amount cut off. It will be noted, however, that in expt. 5 the coleoptile stumps had grown considerably—from 4·5 mm. (6·5 mm. less 2 mm. cut off) to 10·8 mm. Regeneration of auxin production must, therefore, have occurred, since only then could elongation take place.

In a further experiment to check this point sufficient plants were employed to take daily measurements (Table III), and a standard length of 1·5 mm. was on this occasion removed from the coleoptile tip by means of an implement similar to that shown in Fig. 2, but with a deeper channel.

TABLE III

The Growth of the Coleoptile Stump after Deseeding and Decapitation. Experiment 6

			Mesocotyls.	Coleoptile stumps.
3-day-old seedlings			18·0±0·70	5·7±0·13 (46)
Deseeded on the 3rd day	Measured on the 4th day		36·4±0·88	7·1±0·16 (40)
	Decapitated on the 4th day	Measured on 5th day	39·8±1·07	5·9±0·43 (32)
		,, 6th ,,	43·9±0·33	7·4±0·42 (29)
		,, 7th ,,	49·4±1·41	10·2±0·71 (34)
		,, 8th ,,	52·3±0·87	11·4±0·97 (33)

This result corroborates that of expt. 5, and because of the regeneration of an active tip to the coleoptile stump the elongation of the mesocotyl in the absence of coleoptilar auxin cannot by this type of experiment be tested. The results here presented throw some doubt upon the adequacy of deseeding as a method of preventing auxin regeneration in the decapitated coleoptile stumps. Indeed, Skoog's own data are not convincing on this point. In Fig. 4A (on p. 318) Skoog (1936/7) showed the progress of curvature of intact and deseeded test coleoptiles when treated unilaterally with agar blocks containing hormone. The curvature of the deseeded plants increased with time for the first 7 hours, reaching maximum angles which differed with concentration, viz. 58·2° for concentration 1; 40·8° for $\frac{1}{2}$; and 20·5° for $\frac{1}{6}$th, and in the following 13 hours these angles were reduced to 35°, 18°, and 8° respectively. In the case of normal plants which reached their maximum angles in shorter times than mentioned above, the retrogression in curvature which took place was explained, as already discussed, in terms of regeneration of an auxin-producing tip on the side of the coleoptile remote from the block, but no comment was made with regard to the similar reduction found in the curvature of deseeded plants.

Bonner (1933) presented data which showed that regeneration processes occurred in isolated coleoptile segments, and it would appear that regeneration also took place in these deseeded plants, but that its inception was delayed.

The accuracy and validity of the deseeded method for the estimation of small quantities of hormone therefore remains unimpaired, provided the duration of the test is shorter than 7 hours.

3. *Decapitation at the coleoptilar node*

Boyd and Avery (1936), describing the anatomy of the *Avena* seedling, indicated a meristem at the coleoptilar node, but information on its activity is controversial. Araki (1939) concluded that cell division stopped after 2 days' growth, and he explained subsequent elongation of the mesocotyl in terms of cellular extension. On the other hand, Goodwin (1941) recorded that cell numbers in the mesocotyl were nearly doubled between the 2nd and 5th days, while Avery, Burkholder, and Creighton (1939) showed that cell numbers (in a single row) increased from 42·2 in seedlings 42 hours old to 144 in those 86 hours old.

If the growth of the mesocotyl were affected by the activity of this meristem, its removal should result in a sharp decrease in the final mesocotyl length. It was found difficult to cut off the coleoptile exactly at the node in complete darkness, and so a short piece of the mesocotyl was also unavoidably removed. No information is available on the auxin production and regeneration of the exposed mesocotyl cells. The results of two such experiments are recorded in Table IV.

TABLE IV

The Effect of Decapitating Seedlings below the Coleoptilar Node

	Experiment 7.		Experiment 8.	
	Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
3-day-old seedlings . . .	17·4±0·88	5·5 (26)	21·5±0·92	5·7 (35)
Decapitated below the node on 3rd day; stumps measured on 7th day	32·4±2·9(*a*)	— (10)	25·7±0·65(*b*)	— (79)
Controls, not decapitated . .	68·1±1·01	42·0 (29)	66·5±1·41	34·8 (37)
Decapitated *above* node on 3rd day; measured on 7th day . .	57·2±1·17	— (24)	47·2±1·24	— (17)

(*a*) Average of 1 mm. below the node was removed.
(*b*) ,, 2·3 mm. ,, ,, ,, ,, ,,

These results clearly indicate that the removal of the node has a far greater effect in reducing mesocotyl extension than decapitation of the coleoptile. Whether this is due to the removal of an effective meristem, or whether to a disturbance of the auxin relations of the stem apex located immediately above the node, has not yet been decided.

In these experiments an effect, on final mesocotyl length, of the absence of the coleoptile tip was noted for the first time. Some of the seedlings were accidentally decapitated just above the node, and these were measured and have been included in Table IV. It will be seen that these mesocotyls are much longer than those decapitated below the node, but they are, however, significantly shorter than those of the control plants. It was thus thought possible that the effect of removing the coleoptile tip became apparent only when sufficient time was given before measurement for the mesocotyl to terminate its growth. Two further experiments were therefore carried out on 3-day-old dark grown plants, which were decapitated once only, and samples were measured daily and discarded. The results are shown in Table V.

TABLE V

The Effect on Mesocotyl Elongation of a Single Decapitation of the Coleoptile Tip

	Experiment 9. 3-day-old plants (31). Mesocotyls 19·1±0·59 Coleoptiles 5·4.			Experiment 10. 3-day-old plants (46). Mesocotyls 22·8±0·70 Coleoptiles 5·8.		
	Decapitated on 3rd day.	Controls not decapitated.		Decapitated on 3rd day.	Controls not decapitated.	
	Mesocotyls.	Mesocotyls.	Coleoptiles.	Mesocotyls.	Mesocotyls.	Coleoptiles.
Length on 4th day	30·5±0·61 (36)	—	—	31·3±0·81 (18)	37·1±1·06	7·9 (25)
„ „ 5th „	41·7±0·51 (32)	43·2±3·35	9·1 (9)	50·4±1·43 (18)	61·8±1·60	13·0 (23)
„ „ 6th „	47·3±0·88 (34)	53·4±2·87	15·9 (11)	—	—	—
„ „ 7th „	47·9±0·83 (28)	56·6±2·59	21·5 (12)	54·1±1·30 (26)	66·2±1·77	45·0 (21)

Thes eresults confirm those of expts. 7 and 8, and unequivocally show (1) that considerable mesocotyl growth takes place after decapitation, and (2) that the final mesocotyl length is significantly reduced by decapitation.

The possibility that a wound reaction was involved now demanded consideration, and this was tested by the following treatments: (*a*) cutting the tip of the coleoptile vertically for a distance of 2–3 mm.; (*b*) piercing the coleoptile vertically midway between the tip and the node with a narrow blade, for a similar distance. The plants were thus wounded without seriously interfering with the downward path of the hormone, and without removing the tip. The operations were performed in darkness on 3-day-old plants, which were measured when 7 days old. The results are recorded in Table VI, where a significant decrease in final mesocotyl length of the mutilated plants is shown, with no difference in coleoptile length.

TABLE VI

The Effect of Wounding the Coleoptile on the Growth of the Mesocotyl

Experiment 11.	Mesocotyls.	Coleoptiles.
Tip cut at 3 days old; measured on the 7th day .	49·8±0·65	35·2±1·69 (45)
Pierced at 3 days old; „ „ „ „ „ .	50·6±0·75	34·6±1·72 (34)
Controls; measured on the 7th day . . .	57·4±0·9	35·8±1·82 (47)

It seems likely, therefore, that a wound reaction as a consequence of decapitation contributes to the reduction in mesocotyl length in addition to any auxin changes.

This concludes the section of the investigation involving decapitations and the main conclusions drawn are as follows:

1. Mesocotyls of seedlings, in which the auxin content of the coleoptile is maintained at a low level by repeated decapitations for periods of 12½ and

7½ hours, grow, in this period, as much as the mesocotyls of plants not so decapitated.

2. 'Deseeding' does not prevent regeneration of an active auxin-producing tip to the coleoptile stump following decapitation, and in consequence this method does not provide a satisfactory technique for maintaining an auxin-free coleoptile. Mesocotyl growth occurred to the same extent in control and decapitated starved seedlings.

3. The removal of the coleoptilar node reduces the growth of the mesocotyl more markedly than does the removal of the coleoptile tip.

4. Decapitation of the coleoptile reduces final mesocotyl length, but this only becomes apparent when measurements are made after the termination of growth on the 7th day.

5. A wound reaction is also partly responsible for the reduction of mesocotyl length.

4. *Heat treatment of intact seedlings*

Many investigators have reported that heat treatment of cereal seedlings results in a reduction in the final length of the mesocotyl. These include du Buy and Nuernbergk (1929), du Buy (1933), van Overbeek (1936), Inge and Loomis (loc. cit.), and Weintraub and McAlister (1942), and the relevant facts will be briefly mentioned.

Du Buy and Nuernbergk investigated mesocotyl growth with the object of reducing its length without exposing the seedlings to light, to produce straight and robust coleoptiles for the auxin assay test. They found that heating the soil in which the seedlings were growing to 33° C. had the desired effect. Du Buy investigated the effect of temperature on isolated coleoptile tips, which after removal from the plant remained at the experimental temperature, for acclimatization, for 1 or 1½ hours prior to a 1-hour diffusion period into agar blocks. He concluded that production of auxin was completely inhibited at 40° C. (p. 821). Van Overbeek heated intact maize seedlings at 48° C. for ½ hour, and claimed that shorter mesocotyl length was accompanied by a reduced quantity of auxin diffusing from the decapitated coleoptile tip for a period up to 24 hours after treatment. He further showed that the application of indoleacetic acid in lanoline to the seedlings after heating nullified the effects of the treatment, for the lengths of the mesocotyls in these circumstances were then equal to those of the control plants. Inge and Loomis confirmed van Overbeek's observation on intact maize plants, and found that when the emerging coleoptile tips of the seedlings were heated at 51° C. for increasing lengths of time (up to a maximum of 1 hour), then the mesocotyls became progressively shorter. Weintraub and McAlister recorded for *Avena* mesocotyl lengths from 125 mm. at 15° C. falling to 1 mm. at 34° C., but no further details were given.

The results of van Overbeek and du Buy point to the explanation of the reduction of mesocotyl growth in terms of auxin, but there is very little information in the literature on the effect of heat on the length of the coleoptile itself. Inge and Loomis recorded the final measurements of their maize coleoptiles, and these showed a marked reduction in length with increasing exposure to high temperature (51° C.). The growth of the coleoptile is known to be an auxin-controlled process, and its behaviour after heating might therefore contribute useful information to the present discussion.

In the following experiments dark grown plants were used, and they were heated for 3 hours at 40° C. after growing for different periods at normal temperature (22° C.), viz. 2 hours, 19½ hours, 2 days, and 3 days. For each of these 4 treatments 6 sets of plants were grown and after heating they were returned to the incubator. One set was withdrawn daily for measurement (after which it was discarded) from the 3rd to the 8th day after planting.

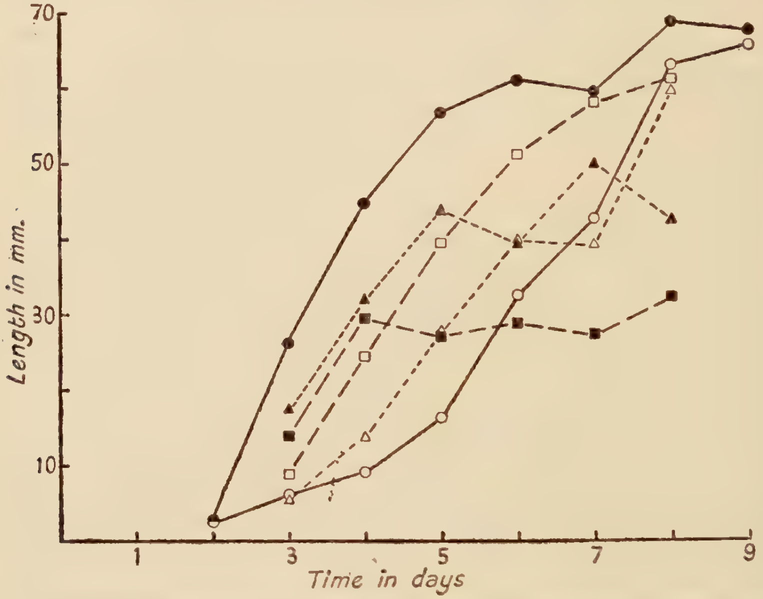

FIG. 3. The effect of heating seedlings at 40° C. for 3 hours, after different periods at normal temperature.

Open circles: coleoptiles Full circles: mesocotyls	Controls. Data for growth in darkness as in Fig. 1.
Open squares: coleoptiles Full squares: mesocotyls	Treatment after 2 days at normal temperature.
Open triangles: coleoptiles Full triangles: mesocotyls	Treatment after 3 days at normal temperature.

Fig. 3 shows the results obtained for heat-treated plants after growth for 2 days and 3 days respectively at normal temperature (expt. 12). The values for the controls are reproduced from Fig. 1 as no separate controls were grown. The results for heating after 2 hours' and 19½ hours' growth at normal temperature are not included; in the former the seeds were heated before they were fully imbibed. Roberts (1948) states that this process takes approximately 4–8 hours; and the growth of the plants was essentially the same as the controls. In the latter, the result corresponded almost exactly with the recorded curves for heat treatment after 2 days at normal temperature.

It is to be noted that the mesocotyl lengths plotted in the graph for the 3rd day include controls (black circles), plants heated on their 2nd day of growth (black squares), and plants unheated at the time of measurement (black triangles). The variation at this time in their lengths are therefore not related to heat treatment but must be attributed to the variability between different sets of seedlings. It has already been stated that the controls were derived from previously obtained data.

The results indicate that as far as the mesocotyls are concerned the treatment may not affect the rate of growth, for the slopes of the three curves initially remain approximately parallel. The high variability of the different sets of seedlings, however, makes this conclusion somewhat uncertain. The effect is seen, however, in the final length of the mesocotyl; for 2 days after treatment growth ceases, and consequently the earlier the treatment the shorter is the mesocotyl. The situation with the coleoptile is very different, for here heat treatment enhances the growth rate. Comparing the three curves it is seen that the growth rate attained by the controls 5–6 days after germination is reached by the heated plants in 3 and 4 days respectively. The marked rise in coleoptile growth follows 2 days after treatment, but in spite of this stimulation final coleoptile length is the same in all cases and is thus independent of treatment (note, however, that the 7th day sample, open triangle, is aberrant).

The results obtained may now be examined from the point of view of the auxin hypothesis. If the higher growth rate of the coleoptile is attributed to the production of more auxin, this must be an after effect of heating, for subsequently all the seedlings were kept at the same temperature. If indeed, more auxin is produced, this extra supply not only fails to reach the mesocotyl (assuming that auxin does regulate its growth) but it is also not reflected in a greater final length of the coleoptile. On the other hand, the final length of the mesocotyl is reduced by heating and the arrest of growth occurs at the time when the coleoptile attains its highest growth rate.

It would be difficult without multiplying subsidiary hypotheses to reconcile these findings with the theory of auxin control of mesocotyl growth.

Van Overbeek (1936) concluded from his experiments that heating maize seedlings reduced subsequent production of auxin in the coleoptile tip, but this does not appear to be likely in the present case; nor does the conclusion of du Buy (1933), that a 2-hour treatment at 40° C. inhibits auxin formation, find any support in these results.

Gregory and Purvis (1945, 1948, and unpublished) have demonstrated that the effect of high temperature on winter rye previously vernalized for short periods could be annulled by a further exposure to low temperature. It was thought of interest to ascertain whether the inhibiting effect of heating on mesocotyl growth could be similarly removed by subsequent chilling.

A factorial experiment was carried out combining three levels of low temperature (0, 3, 6 hours at 1° C. respectively) with two high temperature (0 and 3 hours at 40° C.). The results of expt. 12 showed that heating the seedlings after 2 days' normal growth produced the greatest effect, and this procedure was adopted in the present instance, the low-temperature treatment following immediately. The plants were then returned to 22° C. and measured when 7 days old, and to simplify the statistical calculation, the number of plants in each treatment was reduced by random selection to the lowest number found in any one treatment; in expt. 13, to 41 plants, and in expt. 14, to 48 plants. The measurements in millimetres are recorded in Tables VII and VIII.

In expt. 13 it was noted that the roots of the heated plants appeared to be longer and more robust than those of the unheated plants. In expt. 14, therefore, the main roots (4 or 5 per plant) were also measured, and their total average lengths are included in Table VIII.

TABLE VII

The Effect of Heating and Chilling on the Length of Mesocotyl and Coleoptile. Experiment 13, 41 Replicates

			Low-temperature treatment (1° C.).		
			Control.	3 hours.	6 hours.
High-temperature treatment	Control	Mesocotyls	75·4 ± 1·9	65·9 ± 1·0	66·0 ± 1·4
		Coleoptiles	29·9 ± 2·2	45·7 ± 2·2	37·3 ± 1·8
	3 hours	Mesocotyls	23·3 ± 0·9	17·8 ± 1·8	16·4 ± 1·0
		Coleoptiles	46·6 ± 1·4	45·6 ± 1·8	44·4 ± 1·3

TABLE VIII

Experiment 14, 48 Replicates

			Low-temperature treatment (1° C.).		
			Control.	3 hours.	6 hours.
High-temperature treatment	Control	Mesocotyls	78·7 ± 1·3	71·9 ± 0·94	74·1 ± 1·15
		Coleoptiles	36·2 ± 1·75	39·6 ± 2·1	44·1 ± 1·4
		Roots	138·3 ± 6·7	154·1 ± 10·0	177·1 ± 6·0
	3 hours	Mesocotyls	26·9 ± 0·77	18·5 ± 1·1	18·8 ± 0·81
		Coleoptiles	48·1 ± 1·6	51·5 ± 1·45	51·6 ± 0·91
		Roots	262·5 ± 11·3	275·1 ± 9·9	270·8 ± 9·3

The analysis of variance for these two experiments showed highly significant main effects ($P = 0·001$) of high and low temperature for the mesocotyls. For the coleoptiles both effects were also highly significant (for heating $P = 0·001$; for cooling $P = 0·01$). The interaction of high and low temperature was significant only for the coleoptiles in expt. 13 ($P = 0·01$).

The main results of the experiments may be summarized as follows: (1) the effects of heating are not reversed by subsequent low-temperature treatment;

(2) low temperature augments the effect of high temperature and produces a further slight decrease in mesocotyl length; (3) low temperature produces a slight reduction in mesocotyl length in unheated seedlings; (4) wherever a reduction in mesocotyl length takes place the growth of the coleoptile is stimulated; (5) heat treatment markedly promotes root growth; low temperature has little effect.

In these experiments the average length of the control mesocotyls in some cases is more than 70 mm. In all previous experiments (1–12) the tins used as experimental chambers (see p. 181 on Methods) were 9 in. tall, and the mean final mesocotyl length was 65 mm. This discrepancy was statistically highly significant. In expts. 13 and 14 only the tins used were 7 in. tall, and it was thought that these smaller tins were responsible for the aberrant result. A subsidiary investigation was carried out to test this and led to the discovery of the effect of carbon dioxide concentration on mesocotyl growth mentioned in the introduction.

5. *Treatment of intact seedlings with* KCN *solutions*

Potassium cyanide is one of the most effective respiratory poisons, and Bonner (1936) has shown that elongation of coleoptile segments was markedly reduced by immersion for an 8-hour period in KCN solutions of strengths 1×10^{-3} M and 3×10^{-3} M; they grew 28·9 per cent. and 16·5 per cent. respectively of the elongation of the controls. Since the extension of these segments is an auxin-controlled process, the reduction in length noted indicates that the reagent affected either auxin itself or some intermediary stage in the growth process.

When etiolated *Avena* seedlings are given short exposures to light, a change in mesocotyl and coleoptile growth follows, which is similar to that produced by heating—in both cases the length of the mesocotyl is reduced, and the coleoptile is stimulated. Since the suppression of the mesocotyl is reputed to be due to the destruction of auxin by light, and KCN has been shown to inhibit auxin-linked processes, use was made of KCN in a further analysis of the light effect.

The experiments were carried out using four sets of 3-day-old dark grown seedlings, one for exposure to each of the four following conditions: (1) KCN illuminated; (2) KCN not illuminated; (3) controls illuminated; (4) controls not illuminated. While in darkness the plants were transferred from the tins in which they normally grew to glass jars, which were just large enough to accommodate the crystallizing dishes, and the 57 seedlings could thus be moved as a single unit. The test solution was then poured into the jars completely to submerge the plants; the controls were similarly immersed in tap-water, and the jars were then covered with discs of plate glass. After a pre-treatment period of 1 hour in darkness two sets of plants (one immersed in KCN and the other control) were illuminated for 1 hour (intensity 200 ft.-candles). In this and subsequent experiments where illumination is mentioned, ordinary white light given by opal electric lamps was employed.

Following this treatment the plants were removed from the solutions, and those immersed in KCN were quickly rinsed in tap-water to wash off the reagent. They were then transferred to previously prepared specimen tubes partially filled with fresh tap-water, and thence to the incubator in their tins, where they remained until measured on the 7th day from planting. The control plants were treated similarly except for the rinsing, which was obviously not necessary. All these operations were carried out in darkness; the plants being illuminated only while immersed in the solutions.

Concentrations of 1, 2, 2·5, 3, 4, and 5×10^{-3} M KCN, made up in tap-water, were used in different experiments, and the highest suitable concentration was found to be 2×10^{-3} M, for the percentage of seedlings surviving the 2-hour immersion rapidly fell with increasing concentration; the survival figures corresponding respectively with the above solutions were: 75, 70, 32, 12·3, 15·8, and 0 per cent.

In view of this high mortality after a 2-hour immersion, it seems possible that Bonner's segments were killed by an 8-hour immersion, for he did not return them to water following the treatment to find whether growth were resumed. This possibility seems also to have been noted by du Buy and Olsen (1940).

The results of two experiments using 1×10^{-3} M (41 replicates) and 2×10^{-3} M (40 replicates) are recorded in Table IX.

Table IX

The Effect of Illuminating 3-day-old Plants immersed in KCN *Solutions. Experiments 15 and 16*

		Mesocotyls.	Coleoptiles.
Immersed in KCN (1×10^{-3})	Illuminated	37·2 ± 0·79	37·8 ± 0·95
	Not illuminated	55·0 ± 1·2	40·8 ± 0·86
Immersed in tap-water	Illuminated	35·6 ± 0·78	46·1 ± 1·1
	Not illuminated	49·3 ± 0·86	40·9 ± 1·65
Immersed in KCN (2×10^{-3})	Illuminated	36·5 ± 0·83	39·7 ± 1·04
	Not illuminated	55·8 ± 1·25	34·3 ± 1·12
Immersed in tap-water	Illuminated	34·7 ± 0·8	41·8 ± 1·04
	Not illuminated	52·2 ± 1·0	32·3 ± 1·49

The levels of significance reached by the different factors (*P* values), derived from analyses of variance, are shown in Table X.

Table X

Significance Levels appertaining to Experiments 15 and 16

	Experiment 15 $(1 \times 10^{-3}$ M)		Experiment 16 $(2 \times 10^{-3}$ M)	
	Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
Light *v.* Darkness	0·001	N.S.	0·001	0·001
KCN *v.* Water	0·001	0·001	N.S.	0·01
Interaction	0·05	0·001	N.S.	N.S.

These results show that after immersion in KCN solutions of 1 and 2×10^{-3} M, the plants subsequently grow as well as do those in water, and further, that light produces its normal effect on the mesocotyl. This was also found in a second experiment with 2×10^{-3} M KCN, and in the few plants which survived immersion in 2·5, 3, and 4×10^{-3} M solutions.

If, in fact, KCN inactivates the auxin system of the plant, then it seems unlikely that destruction of auxin by light is the primary process leading to a reduction in mesocotyl length.

6. *Treatment of intact seedlings with iodoacetate*

Further experiments were carried out with iodoacetic acid, which is known to inactivate dehydrogenase systems and enzymes with active —SH groups. Albaum and Commoner (1941) have shown that in very dilute solutions, the most effective being 5×10^{-5} M, this substance affected all the auxin-controlled processes which they studied, in intact seedlings, viz. coleoptile length, root length, and root number. Commoner and Thimann (1941) demonstrated that coleoptile segments totally immersed in 5×10^{-5} M iodoacetate ceased growth after about 17 hours' immersion, while their respiratory activity was reduced by only 9 per cent.

The technique described in the previous section was again employed, replacing KCN by 5×10^{-5} M iodoacetate in all the experiments, with the difference that the plants were immersed when 2 days old, for 17 hours and illuminated for 2 hours at the end of this period. The total immersion was, therefore, 19 hours, and the plants were nearly 3 days old when illuminated. Following the technique of Albaum and co-workers (1941, 1943), distilled water was used throughout the experiment, but under these conditions the plants failed to grow. This was associated with the absence of root growth, for which the presence of calcium ions in the solution is essential.

The experiment was therefore repeated, growing the plants for 2 days in tap-water and then, in darkness, transferring them immediately prior to immersion to specimen tubes containing distilled water with added $CaCl_2$ $6H_2O$ (0·111 g./litre) for the controls, and in addition iodoacetate for the test solution. All solutions were adjusted to pH 6. After illumination the plants were rapidly rinsed and transferred to specimen tubes containing tap-water. When measured on the 7th day the growth of all plants, although very much better than in the first experiment with iodoacetate, was found to be poor. The seedlings were, however, measurable. This adverse effect on growth was probably due to lack of aeration during the long immersion, for Thimann and Bonner (1948) found that coleoptile segments grew better in iodoacetate and organic acid solutions when breaking the surface than when completely immersed. The results recorded in Table XI show clearly that in the illuminated plants the mesocotyl was shorter than in those not illuminated, while the reverse was the case with the coleoptiles.

Beevers and Simon (1949) have shown that iodoacetate is more effective in acid solution, and so in another experiment the pH of the solution in

TABLE XI

The Effect of Illumination on Plants immersed in Iodoacetate Solution. Experiment 17, 30 Replicates

	With iodoacetate.		Without iodoacetate.	
	Illuminated.	Not illuminated.	Illuminated.	Not illuminated.
Mesocotyls	15·0±1·04	28·6±0·97	19·5±1·15	32·7±1·6
Coleoptiles	24·3±1·14	14·8±0·91	27·4±0·99	15·3±1·1

which the plants were grown, and also those in which they were immersed, was adjusted to 4·9 (using dilute HCl). This culture solution (distilled water plus $CaCl_2$ $6H_2O$ 0·111 g./l.) was used throughout the experiment and was changed daily to offset slight alterations in pH which took place. The result is shown in Table XII.

TABLE XII

The Effect of Illumination on Plants immersed in Iodoacetate Solution (pH 4·9). Experiment 18, 29 Replicates

	With iodoacetate.		Without iodoacetate.	
	Illuminated.	Not illuminated.	Illuminated.	Not illuminated.
Mesocotyls	15·1±1·3	30·2±1·81	31·5±1·14	38·0±1·4
Coleoptiles	27·6±0·8	24·4±0·91	32·6±1·24	28·9±1·4

The significance levels reached in expts. 17 and 18 (*P* values), derived from analyses of variance, are shown in Table XIII.

TABLE XIII

Significance Levels appertaining to Experiments 17 and 18

	Experiment 17.		Experiment 18.	
	Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
Light *v.* Darkness	0·001	0·001	0·001	0·01
With iodoacetate *v.* without	0·001	N.S.	0·001	0·001
Interaction	N.S.	N.S.	0·01	N.S.

These two experiments therefore demonstrate that light perception is not prevented by treatment with iodoacetate. In both experiments a significant reduction in mesocotyl length due to the reagent was found, in addition to the light effect, which indicated that penetration into the tissues took place.

7. *The effect of illuminating seedlings under anaerobic conditions*

Van Ameijden (1917) showed that neither geotropic nor phototropic stimuli were perceived under anaerobic conditions when a pre-treatment of 6–8 hours was given prior to stimulation. There was also no reaction when the plants were returned to normal aerobic conditions following stimulation under anaerobic conditions, and it would appear that in these circumstances the auxin system was temporarily rendered inoperative. Bonner (1933) also found that coleoptile segments immersed in a solution of growth-promoting substance elongated only slightly in the absence of oxygen.

In consequence, it was thought to be of interest to find whether seedlings exposed to light while under anaerobic conditions showed a reduction in mesocotyl length. Day-old, dark grown seedlings were used, and a 2×2 factorial arrangement was again employed. Four sets of plants were therefore necessary for each experiment, one for exposure to each of the four following conditions: (1) anaerobic illuminated, (2) anaerobic not illuminated, (3) aerobic illuminated, and (4) aerobic not illuminated. The plants to be exposed to anaerobic conditions were transferred in darkness to specimen tubes containing air-free tap-water, and were then put into the jars which had been previously used for the KCN and iodoacetate experiments. Anaerobic conditions were procured in the jars by enclosing in them a suitable quantity of alkaline pyrogallol. To prevent a reduction of pressure in them due to the absorption of oxygen they were connected to the atmosphere through bubblers also containing pyrogallol solution. Anaerobic conditions were maintained for 7 or 24 hours, with illumination (30 ft.-candles) during the last 5 or 10 hours. After the exposure to light the plants were transferred in darkness to their respective tins and put into the incubator, where they remained until measured on the 7th day. The results of two experiments are recorded in Tables XIV and XV.

TABLE XIV

The Effect of Illuminating Seedlings under Anaerobic Conditions. Experiment 19, 42 Replicates

7-hour anaerobiosis; illumination for final 5 hours.

	Anaerobic.		Aerobic.	
	Illuminated.	Not illuminated.	Illuminated.	Not illuminated.
Mesocotyls	44·2±0·706	53·8±0·703	43·3±0·854	55·4±0·847
Coleoptiles	61·1	59·9	65·3	59·1

TABLE XV

The Effect of Illuminating Seedlings under Anaerobic Conditions. Experiment 20, 40 Replicates

24-hour anaerobiosis; illumination for final 10 hours.

	Anaerobic.		Aerobic.	
	Illuminated.	Not illuminated.	Illuminated.	Not illuminated.
Mesocotyls	37·5±0·755	51·7±1·07	26·4±0·82	66·7±1·03
Coleoptiles	49·2	40·4	60·7	56·9

Analyses of variance were carried out on the mesocotyl data for these two experiments, and the significance levels reached are recorded in Table XVI.

Table XVI

Significance Levels reached in Experiments 19 and 20. (Mesocotyls only)

	Experiment 19.	Experiment 20.
Light *v.* Darkness	0·001	0·001
Aerobic *v.* anaerobic	N.S.	0·05
Interaction	N.S.	0·001

There can be no doubt that light was perceived by the seedlings under these conditions, leading again to the conclusion that auxin is not a necessary factor in the perception process.

The results presented to this point have been concerned directly with the auxin relations of the growth reactions of the mesocotyl. Additional experiments not immediately concerned with this problem, but designed to provide further information on the physiology of the seedling, have been carried out and are recorded here.

8. *The illumination of plants at low temperature*

The results of section 4 indicated that chilling caused a reduction in mesocotyl length, and it was considered of interest to find if light were perceived by seedlings maintained under these conditions during illumination.

In expts. 21 and 22, 2 sets of plants were grown in darkness for 3 days at 22° C. and were then placed in a refrigerator for 2 hours to reach the prevailing temperature of 2° C. One set was then illuminated by a water-screened lamp (200 ft.-candles) in the refrigerator for 24 hours, the other set remaining in darkness as controls. The plants were then grown for a further 5 days at 22° C., when they were measured; the results of the two experiments are recorded in Table XVII.

Table XVII

The Effect of Illuminating Plants at 2° C. Replicates in Brackets

	Illuminated.		Controls.	
	Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
Expt. 21	10·7±0·7	60·9±0·75 (40)	30·7±0·66	70·0±0·85 (40)
Expt. 22	26·2±1·06	72·6±0·99 (42)	36·8±1·43	74·6±1·64 (36)

There is little doubt that illumination had produced its usual effect on the mesocotyl. The effect of low temperature, however, was striking in that the coleoptile lengths were similar, and on average slightly longer than those of normal plants. The control mesocotyls were, on the other hand, much shorter than normal controls and this effect of low temperature was further investigated.

Six sets of seeds were sown and immediately put into the refrigerator at 1° C. Subsequent treatment is detailed in Table XVIII.

TABLE XVIII

The Effect of Low-temperature Treatment of Seeds on the Length of the Mesocotyl. Experiment 23

	Mesocotyls.	Coleoptiles.
3 days at 1° C.; 7 days at normal temperature	61·2±0·98 61·4±0·84	52·6±2·02 (49) 56·1±1·87 (51)
7 ,, ,, ,, ,,	53·1±0·97 49·2±1·13	60·1±1·64 (55) 57·1±1·48 (46)
14 ,, ,, ,, ,,	37·8±0·96	68·3±0·69 (55)
14 ,, 1 day at normal temperature followed by 1 further day at 1° C., and then 6 days at normal temperature	26·4±1·04	57·6±0·84 (50)

It will be noted that in expts. 21 and 22 the 26-hour treatment at 2° C. reduced the length of the control mesocotyls to 30·7 and 36·8 mm. respectively. In expt. 23, 14 days at 1° C. were necessary to produce a similar result. The object of the treatment given to the second set of seeds chilled for 14 days was to determine whether they were more sensitive to low temperature after a short period of growth at normal temperature. The result indicates that this was so.

There is shown, in expt. 23, a progressive reduction in the length of the mesocotyl with increased chilling period, and apart from the last measurement a progressive lengthening of the coleoptile. It would appear, therefore, that the mechanism controlling mesocotyl extension is sensitive to low temperature. The smaller final length of the mesocotyl is not due to the fact that its growth was incomplete at the end of the experiment. So far as the coleoptile is concerned, low temperature has little effect on final length and any auxin changes presumed to occur to account for the shortness of the mesocotyl are not apparent from the behaviour of the coleoptile.

In their investigation on vernalization, Gregory and Purvis (1938) treated winter rye at 1° C. for periods up to 6 weeks, and it was decided to grow plants for this period at low temperature and find whether they were then sensitive to light. The seeds were sown as usual, and grown for 6 weeks at 2° C., and then one set was illuminated for 24 hours (100 ft.-candles). The plants at this time were observed to be approximately 2 cm. long, and so almost equivalent to normal 3-day-old plants. After exposure the plants, illuminated and controls, were transferred to the incubator and were measured after a further 6 days' growth. The results of two such experiments are shown in Table XIX.

TABLE XIX

The Effect of Illuminating Seedlings grown for 6 Weeks at 2° C.

	Illuminated.		Not illuminated.	
	Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
Expt. 24	17·2±0·60	64·1±0·71 (69)	45·5±0·79	78·7±0·59 (65)
Expt. 25	26·1±0·93	64·6±0·76 (47)	53·9±1·23	77·3±0·83 (62)

There is no doubt that the seedlings were still sensitive to light and responded in the normal manner. Further work is required to analyse this effect.

9. *The illumination of excised embryos*

Schander (1934), Cholodny (1935), and De Ropp (1939) have shown that during the first few hours of soaking, substances which affect the growth of the cereal embryo are transferred to it from the endosperm. There is thus the possibility that metabolites such as these may be concerned with the production of the light-sensitive materials in the seedling. To test this point, embryos were dissected from sterilized dry grain (Purvis, 1948), and planted in tubes containing 5 c.c. of White's nutrient (1934) with 1 per cent. agar added, and adjusted to pH 6·5. They were grown for 48 hours in darkness in the incubator, and then half of the total number planted were exposed to light for 5 hours (200 ft.-candles). Following this treatment they were grown on for a further 18 days at 22° C., in darkness, when they were measured. The results of two experiments are recorded in Table XX.

TABLE XX

The Illumination of Excised Embryos

	Illuminated.		Not illuminated.	
	Mesocotyls.	Coleoptiles.	Mesocotyls.	Coleoptiles.
Expt. 26 . .	4·4	18·6 (9)	18·8	18·9 (12)
Expt. 27 . .	7·8	17·5 (21)	18·2	13·6 (21)

The reduction in mesocotyl length as a result of illumination indicates that the embryos had developed the necessary perceptive and responsive systems, without any special metabolites or growth-stimulating substances derived from the endosperm.

10. *The amino acids of etiolated and illuminated seedlings*

The methods of chromatographic analysis (Consden, Gordon, and Martin, 1944) are suitable for the examination of plant material for amino acids, and 3-day-old seedlings, some illuminated for 5 hours on the 3rd day and controls not illuminated, were so analysed by Mr. F. J. Richards of this Institute.

The result showed that there was no difference in the free amino acid content between the two sets of plants, and their concentration, as judged by the depth of colour produced by the Ninhydrin reagent, was approximately the same.

Those present in sufficient quantity to permit detection by this method were: aspartic acid, glycine, alanine, valine, leucine, threonine (trace only), and tyrosine (trace only), together with the amides glutamine and asparagine.

11. *Experiments on drying down germinated grain*

Milthorpe (1950) has shown that wheat seedlings will survive complete desiccation even after considerable growth has been made, and it was thought of interest to find whether the changes which take place in a seedling upon illumination would persist and become manifest when the grain was subsequently germinated in darkness.

In these experiments grain was germinated for 24 hours in darkness at 22° C. and then some were exposed to light (200 ft.-candles) for 5 hours, while others remained in darkness as controls. After illumination both sets of grain were dried superficially on blotting-paper and enclosed in a desiccator over calcium chloride, where they remained in darkness until replanted. They were then allowed to grow for the usual period of 7 days, when they were measured.

In the first of these experiments some of the illuminated seeds were grown on immediately in darkness, to provide data on the effect of illumination on seedling growth unaffected by the drying treatment. Samples of the dried grain were planted on two occasions, after 3 and 14 days in the dry condition. In the second experiment the germination and illumination treatment was the same as before, but on this occasion only one planting was made; after 11 days in the dry condition. The results are recorded in Table XXI.

TABLE XXI

The Effect of Drying Down Germinated Grain

		Mesocotyls.	Coleoptiles.
Illuminated: not dried down		40·2	54·4 (26)
Dried down for 3 days	Illuminated	29·2	50·7 (26)
	Controls	43·4	57·2 (22)
Dried down for 14 days	Illuminated	30·4	23·0 (9)
	Controls	39·1	46·4 (11)
Dried down for 11 days	Illuminated	29·1	51·3 (54)
	Controls	39·0	52·1 (46)

These results indicate that the effects of illumination have survived the drying period. It would also appear that drying itself caused a reduction in subsequent growth approximately equal to that of illumination, to which the light effect was added in the exposed plants.

In a further experiment in which illumination prior to drying down lasted only 3 hours, the lengths of the mesocotyls of the control and treated plants were the same (41·4 mm.). The effect of drying was thus evident, but for some unknown reason the light effect was missing. In view of the extreme sensitivity of the seedlings it is improbable that the duration of illumination was too short, and this result stands discrepant.

IV. DISCUSSION

It is now well established that as a result of illuminating etiolated *Avena* seedlings final mesocotyl length is reduced, but the explanation of this phenomenon in terms of auxin destruction by light has recently been questioned by a number of workers. Goodwin (1941), who showed that the light response could be divided into an initial rapid phase, and a later slower one, considered that auxin might be concerned with the latter but not with the former process. Schneider (1941) demonstrated that mesocotyl segments immersed in a solution of auxin, which was thus in ample supply, were shorter following illumination by dim red light than were the controls not so illuminated. He was unable to find any difference in the amount of auxin diffusing from the cut-off tips of dark grown seedlings and those illuminated by dim red light up to 75 hours of age, a result which is at variance with van Overbeek's work (1936). Again, Galston and Hand (1949*b*) have shown that the amount of auxin in illuminated pea epicotyls was the same as in dark controls, and they consider that the reduction in length which takes place during illumination is due to a change in the sensitivity of the cells to auxin. As a result, however, of the observation that auxin is photocatalytically decomposed by riboflavin (Galston 1949), Galston and Baker (1949) have now concluded that the reduction in length of pea epicotyls is due to the decomposition of auxin by light in the presence of riboflavin. This would appear to contradict the finding mentioned above that free auxin content is unaffected by illumination. The riboflavin reaction is insensitive to light frequencies above 5,200 Å, but has a maximum activity at 4,400 Å; on the other hand, mesocotyl growth is reduced most effectively by light of wave-length 6,300–6,600 Å, the effect of radiation falling rapidly to a very low level between 4,000 and 5,000 Å (Weintraub and Price, 1947). It seems clear, therefore, that the riboflavin deactivation of auxin is not necessarily of major importance in the reaction of the mesocotyl to light.

The data presented in Fig. 1 also raise a difficulty in the way of acceptance of the control of mesocotyl growth by the auxin diffusing from the tip of the coleoptile as postulated by van Overbeek (1935), for initially, when the mesocotyl is growing at its maximum rate, coleoptile growth is slow despite the fact that the auxin transmitted to the mesocotyl must on this hypothesis have passed through the coleoptile tissues. There is, further, an inverse relation in the growth of the two parts of the seedling, noted in many of the results presented here, that when the mesocotyl is growing rapidly, the coleoptile elongates slowly and only increases its rate of growth when the extension of the mesocotyl is almost complete. The cause of this reciprocal relation is still obscure, but the evidence available seems to point to the coleoptilar node as the seat of control. Araki and Hamada (1937) found that while local illumination of the coleoptile tip produced an effect on subsequent mesocotyl growth, local illumination at the node had a far greater effect. It has here been shown that removal of the coleoptile tip does not have as much effect on mesocotyl growth as had previously been supposed, and where such an effect was found it could be associated with a wound reaction. On the other hand, removal of the node produced a marked reduction in mesocotyl growth and it thus seems possible that the nodal complex together with the plumular growing-point has some connexion with mesocotyl elongation. Auxin control of growth is thus not precluded.

The results of the experiments involving potassium cyanide, iodoacetate, and anaerobiosis, all of which are known to inhibit auxin activity, indicate that the perception of light is a separate phenomenon from the later growth response of the seedling, and that auxin as such is not a participant in the light absorption process. In geotropism and phototropism, where auxin is known to be the reactant, then anaerobic conditions prevent the perception of the stimulus. It would appear, therefore, that the energy accepted by the seedlings during illumination, while exposed to KCN, iodoacetate, and anaerobic conditions, was passed on to the growth-controlling mechanism after return to aerobic conditions. The separation in time of energy acquisition and its utilization is not unknown, for Vogler (1942) found that *Thiobacillus thioöxidans* could in the absence of CO_2 build up a store of energy by oxidizing sulphur, and subsequently could utilize this energy to assimilate carbon dioxide supplied to it in the absence of an oxidizable substrate.

The results of the high-temperature experiments are also difficult to interpret in terms of auxin destruction because of the increase in the growth rate of the coleoptile and the delayed response of the mesocotyl. It is true, however, that the total length of the treated seedlings is less than the total length of the controls, and so the final effect might be cited as evidence for the destruction of auxin or of any other growth-promoting metabolite. Indeed, Galston and Hand (1949*a*) have recently sought to explain the reduction in length of pea epicotyls, following treatment at 35° C. and above, in terms of a thermal destruction of adenine, for the addition of small quantities of this substance to the culture solution overcame the diminution in the growth of the segments, in the same way that van Overbeek (1936) found that indoleacetic acid in lanoline similarly removed the effect of heating in maize seedlings. In view of the wide distribution which adenine is likely to have in plant cells, it seems difficult to explain the inverse growth of the different parts of the seedling, or how it is that the effects of heating are confined to the mesocotyl, without postulating further hypotheses.

The effect of low temperature on the growth of *Avena* seedlings is not immediately concerned with the auxin theory, for although du Buy (1933) found less auxin diffusing from cut-off coleoptile tips maintained at low temperatures, and van der Weij (1932) showed that the quantity of auxin transported through coleoptile tissue was less at low than at higher temperatures, no claim for the destruction of auxin by chilling has been made. But this clearly causes an effect similar to both high temperature and light. The results presented, however, have a bearing on the suggestion of Weintraub and McAlister (1942), recently revived by Goodwin and Owens (1948), to the effect that protochlorophyll may act as the photoreceptive substance. It is evident that, whatever the substance, it must be present in the mesocotyl in view of Schneider's (1941) finding that isolated segments respond to light in the absence of the coleoptile. Thus the reaction does not always depend on the transference of a stimulus to the mesocotyl from the coleoptile, although this may occur where the mesocotyls of seedlings in the soil cease to grow when the emerging coleoptile tip is illuminated. How far this is the transference of a stimulus or the transmission of light through the transparent tissues has not finally been decided. In the writer's experience, the mesocotyls of seedlings grown in darkness and later exposed to light do not assume a green colour although the leaves and coleoptile rapidly do so. This may mean either that protochlorophyll is absent from the mesocotyl or is present there in such small quantities that upon exposure only an imperceptible amount of chlorophyll is produced from it. Smith (1948) has shown in barley seedlings germinated at normal temperature, but which, after an acclimatization period of 2 hours, were maintained at 0° C.±1·5° during prolonged illumination, that chlorophyll was formed rapidly from the protochlorophyll synthesized in the initial development of the plants at normal temperatures, and that its amount gradually fell when illumination was continued. The duration of the experiment was 40 hours, and at the end of this period chlorophyll content had fallen to approximately half of the maximum, which was reached in about 2 hours after the beginning of illumination. The observed fall was explained as resulting from two processes: firstly the destruction of chlorophyll by light, and secondly the absence of its resynthesis due to the inhibition of protochlorophyll formation by low temperature. If this is indeed the case, then presumably the seedlings grown for 6 weeks at 2° C. (expts. 24 and 25) contained no protochlorophyll, but they were yet sensitive to light. It would be of immediate interest, therefore, to have information on the protochlorophyll content of etiolated mesocotyls, and of plants germinated for 6 weeks at low temperature before any decision on the role of protochlorophyll in the perception mechanism can be reached.

An attempt was, however, made to assess the possible role of protochlorophyll in the reaction by illuminating 3-day-old plants through chlorophyll solutions, acting as filters, prepared from fresh grass cuttings. The experiments were based on the supposition that a chlorophyll solution would absorb just those frequencies to which the receptor in the plant would be responsive; a reaction by the seedling was therefore not expected. A reduction in the length of the mesocotyl was recorded in two experiments after only a 1-minute exposure, as well as in others with longer exposures, but the results are not considered to be definitive on two grounds: (*a*) there is the possibility that the effect was due to white light transmitted to the plants through the glass walls of the cell containing the solution, and (*b*) the absence of a reduction in mesocotyl length following exposure through a chlorophyll solution cannot finally identify protochlorophyll as the receptor because of differences in the spectra of the two substances (Koski and Smith, 1948). It would not be impossible to circumvent the first difficulty, but the second demands the illumination of the plants through a protochlorophyll solution, and the difficulty of the preparation has so far precluded this experiment being done.

The intention in this discussion has been to discriminate sharply between the two aspects of the problem, viz. the perception of the light stimulus by the seedling, and the resultant growth reaction.

It has been shown that conditions known to inhibit auxin effects do not interfere with light perception. The effect of illumination on the subsequent growth of the mesocotyl may be due either to the production of some specific inhibitor, or the destruction by light of some activator or its precursor already present before illumination. So far as the first suggestion is concerned, the difficulty is the fact that illumination of the coleoptile leads to a reduction in growth of the mesocotyl, which indicates a non-localized effect. It may be pointed out, however, that it would be extremely difficult to prevent transmission of light in the translucent coleoptile from reaching the mesocotyl, especially as the effective intensity needed is very low.

Evidence for the production and persistence of some inhibitor was sought in this work and the results of the drying-down experiments (section 11) may be explained on this supposition. Equally they would be consistent with the view that some metabolite essential for growth, which the embryo was unable to resynthesize during the second germination, was destroyed by illumination and by the drying treatment. This presents the same difficulty that other similar hypotheses encounter, namely, how to explain the sensitivity of the mesocotyl to a treatment which hardly affects the coleoptile.

With regard to the destruction of an activator, attention has been confined to the possible role of auxin. The evidence presented definitely excludes the primary effect of coleoptilar auxin in the reaction. This in no way disposes of an auxin hypothesis, for it is always possible that auxin is delivered to the mesocotyl from the growing-point of the plumule situated just above the node. The likelihood of an auxin precursor reaching the mesocotyl from the endosperm may be excluded on the grounds that the embryo separated from the dry grain behaves in every way as the normal plant.

Thus it may be concluded that the elongation of the mesocotyl is not controlled by auxin diffusing downward from the coleoptile tip, and that auxin is not the primary receptor in the perception process. The conclusion reached by Schneider (1941), that '. . . the reciprocal light-growth relationship of the mesocotyl and coleoptile is made up of two oppositely directed and independent responses . . .', finds much support in the data presented here.

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Influence of the Numbers of *Rhizobium* supplied on the Subsequent Nodulation of the Legume Host Plant

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With two Figures

ABSTRACT

The effect of numbers of *Rhizobium* supplied on the subsequent numbers of nodules formed was tested with *Phaseolus radiatus* var. *aurea* in water culture.

Nine weeks after inoculation plants supplied with from 88,700 to 887,000 bacteria per c.c. of culture solution bore significantly more nodules than those given from 89 to 887 per c.c. An inoculum of 44,350 per c.c. produced intermediate numbers of nodules.

One week after inoculation, the percentage of curled and of infected root-hairs on the seedlings was roughly proportional to the logarithm of the bacterial numbers supplied, while the numbers of nodules produced at this stage already showed a significant difference between extreme doses of inoculum.

Nine weeks after inoculation the final nodule numbers were related to the percentages of curled and of infected root-hairs after one week.

The final nodule numbers are related to the growth of the root system since the final number of nodules per gramme of root is independent of the initial dose of inoculum, the effect of which was mainly to cause an increase in root growth.

INTRODUCTION

THE factors controlling the invasion of the roots of a higher plant by a micro-organism are so complex in their interactions as to make it very difficult to determine the effects of any one factor. The nodule bacteria (*Rhizobium*) of the Leguminosae provide a field of study that has special advantages in studying this problem. The multiplication of the bacteria in the root surroundings can be followed, while the effect on this of such factors as soil conditions and root secretions, as well as the behaviour in soil of different strains of *Rhizobium*, alone and in competition, can be studied. The mode of entry, through the root-hairs, enables the primary infection to be separated from formation of nodules by the bacteria and both can be estimated quantitatively by counts. Finally, the growth and persistence of the bacteria in the nodule tissue can be observed and related to their activity as measured by the amount of nitrogen fixed.

There is evidence that under satisfactory growth conditions for plant and bacteria the maximum number of nodules developed on a given mass of roots is limited by factors inherent in the bacterial strain (Chen, 1941), and also by the host plant, in which genetical factors can alter the number of nodules produced by a given bacterial strain (Nutman, 1946). Within the limits set

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by such inherent factors, however, conditions of the environment can greatly affect the number of nodules (Wilson, 1917). The environment can directly influence the multiplication of the bacteria in root surroundings or their movement through them (Thornton and Gangulee, 1926), or its influence on nodule formation may be indirect through a change in the physiology of the plant (Brenchley and Thornton, 1925; Thornton, 1935).

With such a complex of factors, inherent and external, that may influence nodule numbers, it is an interesting question how far the initial dose of bacteria supplied to the root surroundings affects the numbers of nodules developed; and whether, in particular, multiplication of the bacteria outside the roots, in conjunction with the upper limit of nodule numbers as set by factors inherent in the plant, will even out any effects of the initial bacterial dose.

This question has some importance for the practice of legume inoculation and bears on the problem of competition for nodule production between related strains when more than one is present.

There is inadequate evidence at present as to the effect of initial dose of inoculum. Perkins (1925) in an experiment with soybeans grown in sand from seed treated with a range of dilutions of bacterial suspension found that the nodule numbers were increased as the inoculum rose from 1 to 50 bacteria per seed, but that more bacteria than this had no further effect. Thornton (1929), in a field trial with lucerne, found that an increase in inoculum of from 2,500 to 20,000 bacteria per seed resulted in an increase from 170 ± 13 to 238 ± 15 nodules per 100 plants during 2 months' growth of the crop, but low rates of inoculum were not tried.

The work here described was designed to test the correlation between the initial dose of bacteria supplied and the number of nodules developed on *Phaseolus radiatus* var. *aurea* Prain, commonly known in India as 'sona mung', using a pure culture of a single strain of *Rhizobium* isolated from the nodules of this plant.

DESCRIPTION OF THE EXPERIMENT

Seeds were externally sterilized with 0·4 per cent. $HgCl_2$, washed in sterile water, and germinated in sterile wide test-tubes lined with moist filter-paper. The seedlings were transferred aseptically to sterilized culture-tubes, each receiving one plant. Each tube, $1\frac{1}{2}$ in. diam. and 8 in. long, contained 75 c.c. of nitrogen-deficient culture solution having the following composition: K_2HPO_4, 0·5 g.; KH_2PO_4, 0·5 g.; $MgSO_4$, 0·2 g.; NaCl, 0·1 g.; $Ca_3(PO_4)_2$, 2·0 g.; $FePO_4$, 0·5 g.; $FeCl_3$, 0·01 g.; distilled water, 1 litre.

Each culture-tube was previously lined inside with a strip of narrow, folded, and stiff filter-paper up to the height of water-level in the tube, to support the plant.

A suspension of *Rhizobium* from *Phaseolus* root nodules containing $10 \cdot 5 \times 10^7$ bacterial cells per c.c. was prepared by haemocytometer counts. The suspension was diluted to varying degrees for the inoculation of 7 sets of culture-tubes. The number of bacteria are shown in Table 1. On the left are the bacterial numbers aimed at, on the right the actual number of viable cells as determined by plating the thickest suspension on yeast extract agar medium.[1]

TABLE I

Scheme of Number of Bacteria in Treatment Sets

Bacterial number (Haemocytometer count) per c.c. in root surrounding.	Symbols used for different treatment sets.	Viable bacterial number (plate count) per c.c. in root surrounding.
1×10^6	A	887,000
5×10^5	B	443,500
1×10^5	C	88,700
5×10^4	D	44,350
1×10^4	E	8,870
1×10^3	F	887
1×10^2	G	89
(Control) 0	H	0

Sixteen replicate plant cultures were inoculated on April 26, 1948, with 1 c.c. of each dilution according to the scheme shown above, when seedlings were 21 days old. One set of 18 cultures was kept uninoculated (control), and remained without nodules until the end of the experiment.

Owing to the loss of cultures by fungal infection only 9 replicates in treatment set 'D' and 10 replicates in some other treatments were available for the final examination. In some treatments one or more cultures were rejected by random selection to bring the number of replicates to 10.

The cultures were examined and nodules were counted at weekly intervals without removing the plants from the culture-tubes. These values are entered in Table II.

TABLE II

Mean Nodule Numbers and their Standard Errors

Treatment sets and bacterial numbers.	n = degrees of freedom.	M. = mean S.E. = standard error.	Dates of nodule count, May and June 1948.								
			3/5	10/5	17/5	24/5	31/5	7/6	14/6	21/6	28/6*
A	9	M.	1·5	1·9	3·8	7·0	9·1	12·6	15·2	18·6	26·8
1×10^6		S.E.	0·34	0·31	0·36	0·67	0·57	0·75	1·06	1·20	2·62
B	9	M.	1·0	1·5	2·5	6·5	9·4	11·5	14·6	19·1	26·3
5×10^5		S.E.	0·21	0·22	0·34	1·22	0·78	0·97	0·91	0·87	2·29
C	9	M.	1·4	2·0	3·9	6·4	9·4	13·4	17·5	21·7	28·5
1×10^5		S.E.	0·27	0·26	0·38	0·76	1·16	1·46	2·60	3·19	3·96
D	8	M.	1·3	1·9	3·9	5·6	8·4	11·6	14·8	19·0	22·1
5×10^4		S.E.	0·24	0·26	0·51	0·80	0·67	0·91	1·63	1·53	3·14
E	9	M.	1·2	1·9	3·9	5·6	7·0	8·4	10·1	14·8	18·6
1×10^4		S.E.	0·24	0·23	0·50	0·62	0·60	0·50	0·60	1·70	2·31
F	9	M.	1·2	1·6	2·9	6·1	7·6	8·9	10·9	14·5	19·6
1×10^3		S.E.	0·20	0·22	0·46	0·80	1·17	0·97	1·21	1·12	2·26
G	9	M.	0·4	1·3	2·1	4·3	6·2	8·1	11·2	13·6	17·2
1×10^2		S.E.	0·16	0·15	0·26	0·47	0·57	0·60	0·57	0·69	1·07

* The last column nodule numbers have been counted after removing the plants from the culture-tubes. The treatment set H, Control, remained without nodules until the end of the experiment.

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[1] This medium had the following composition: Mannitol, 10 g.; $CaCl_2$, 0·2 g.; K_2HPO 0·5 g.; $FeCl_3$, 0·01 g.; $MgSO_47H_2O$, 0·1 g.; NaCl, 0·2 g.; yeast extract, 100 ml.; agar, 20 g. water 900 ml.

At the time of the final harvest the nodules were counted both through the glass of the tubes, in a manner similar to previous counts, and also after removal of plants from the tubes.

The values thus obtained are entered in Table III, together with a *t* test of the differences between the counts made before and after removal.

TABLE III

Significance of Mean Nodules before and after removing Plants from the Culture-tubes of Harvest

Treatment and bacterial number.	Mean nodules before removing with S.E.	Mean nodules after removing with S.E.	*t*.	*n* degrees of freedom.	Probability
A 1×10^6	25·0±1·86	26·8±2·62	1·462	9	0·1–0·2
B 5×10^5	26·6±2·10	26·3±2·29	0·271	9	0·7–0·8
C 1×10^5	26·5±3·36	28·5±3·96	1·793	9	0·1–0·2
D 5×10^4	22·9±2·95	22·1±3·14	0·590	8	0·5–0·6
E 1×10^4	18·0±2·03	18·6±2·31	1·202	9	0·2–0·3
F 1×10^3	20·2±2·09	19·6±2·26	0·896	9	0·3–0·4
G 1×10^2	17·0±0·98	17·2±1·07	0·180	9	0·8–0·9

The values of *P* entered in the last column show that no significant error arises from counting without previously removing the plants from their tubes. At this time also samples of the medium from two tubes selected at random from treatments A and F were plated out to estimate the bacterial numbers present at the end of the experiment. The values obtained are shown in Table IV.

TABLE IV

Final Nodule Counts and Mean Numbers of Viable Bacteria in the Culture Media Sets A and F

Treatment set and bacterial numbers added.	Tube No.	Nodule No.	Bacterial numbers by plate count.	Mean nodules and its S.E.	Mean bacterial number and its S.E.
Set A Bact.—1×10^6	I	26	546×10^7	24±2·0	$(617 \pm 71{\cdot}0) \times 10^7$
	II	22	688×10^7		
Set F Bact.—1×10^3	I	21	140×10^7	19±2·0	$(122 \pm 18{\cdot}1) \times 10^7$
	II	17	104×10^7		

At harvest the tops and roots of the plants were separately dried and weighed. These values are presented in Table V.

DISCUSSION OF RESULTS

Nodulation rates in the various treatments

The curves of nodule numbers for the various sets with differing initial bacterial numbers are shown in Fig. 1. All the curves show a gradual increase in nodule number with time as may be expected.

TABLE V

Mean Nodule Numbers at Harvest and corresponding Mean Dry Weights of Tops and Roots and S.E.s

Treatment sets and bacterial number added.	Mean nodule numbers (1).	Mean dry weight (2) tops (mg.).	Mean dry weight (3) roots (mg.).
A 1×10^6	26·8±2·62	1374·8±153·9	408·2±41·7
B 5×10^5	26·3±2·29	1262·6±93·8	354·2±34·1
C 1×10^5	28·5±3·96	1274·3±137·7	342·0±44·4
D 5×10^4	22·1±3·14	1187·4±125·2	337·4±34·4
E 1×10^4	18·6±2·31	1034·2±117·9	327·5±29·2
F 1×10^3	19·6±2·26	1011·0±113·2	287·4±32·6
G 1×10^2	17·2±1·07	830·1±104·8	277·7±27·6

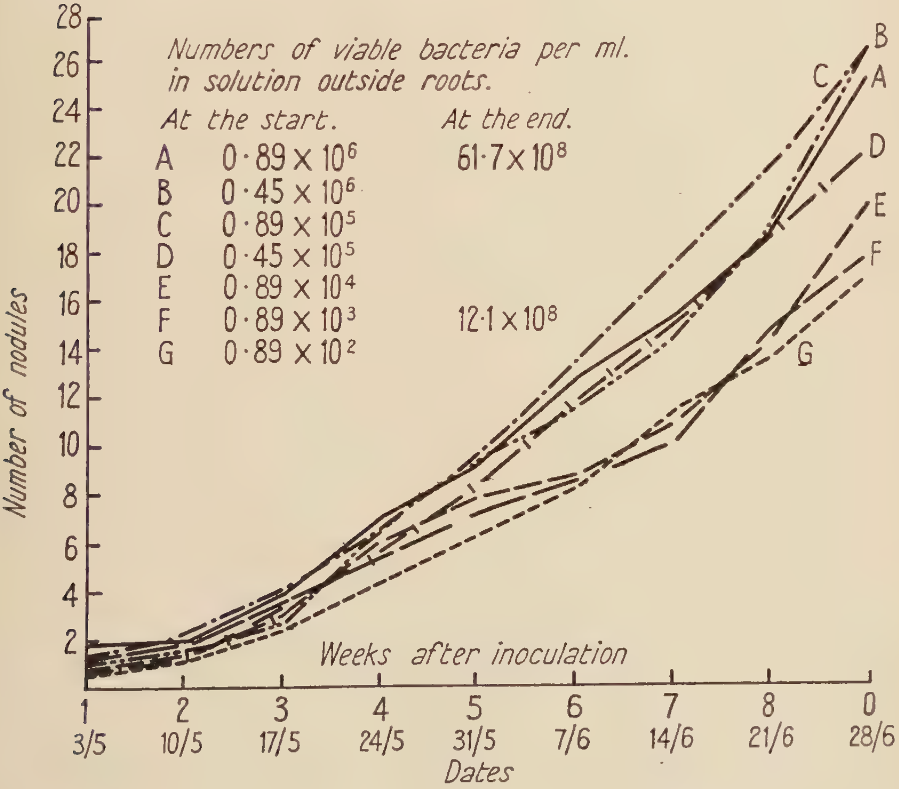

FIG. 1

After the first 7 days divergence begins between the slopes of the curves in the more extreme treatments, and these differences in rate of nodulation are maintained. In consequence, at the end of the experiment the curves fall into two main groups—treatment A, B, C, and treatment E, F, G—D is intermediate.

The relation between final nodule number and original bacterial numbers is seen from the entries in Table II. Analysis of variance was carried out to test the differences in nodule numbers in the various treatments, and it was found that the difference between treatments is significant. Treatments A, B, C do not differ among themselves, nor do treatments E, F, G, but the two groups as a whole are very significantly different (see Table II).

Relation of root-hair curling and infection to the original bacterial numbers in the medium

One week after inoculation counts were made of the percentage of root-hairs curled and infected in the different treatments. The results are shown in Table VI.

TABLE VI

Mean Percentage Numbers of Curled and Infected Root-hairs 7 Days after Inoculation

Treatment set and bacterial number added.	Types of root-hairs	
	Curled.	Infected.*
A 1×10^6	19·5±4·86	7·0±2·08
B 5×10^5	18·5±4·60	5·0±1·67
C 1×10^5	19·5±2·88	7·0±1·53
D 5×10^4	12·5±2·39	4·5±1·57
E 1×10^4	9·0±1·94	3·5±1·50
F 1×10^3	14·0±3·19	5·5±2·41
G 1×10^2	2·5±1·54	1·0±0·67
H 1×0	0·5 —	0 —

* Infected root-hairs, all being curled, are included in the column for curled root-hairs.

The salient point appears that the original bacterial numbers in the cultures are related to the percentage of curled and of infected root-hairs. The number of infected root-hairs is an almost constant fraction of the curled root-hairs, and is roughly proportional to the log. of the bacterial numbers.

Factors controlling final nodule numbers

The data in Table II show further that the degree of divergence in nodule number between treatments (relative number of nodules) remains the same throughout the development of the plant. The final nodule number is more related to the growth of the plant than to the number of bacteria supplied at the beginning or found at the end of the experiment. Indeed, as the data in Table IV show, the bacterial numbers in the various cultures have to a great extent levelled up by the end of the experiment, indicating that later in growth the number of nodules produced is limited by some factor operating from an early stage of growth.

This conclusion is supported by the fact that the numbers of nodules found at the end of the experiment are correlated with the percentages of infected root-hairs found after 1 week (Tables II and VI and Fig. 2).

The clue to the relation of nodule numbers at the beginning and end of the experiment is indicated by the data in Table V. It is there seen that the final weights of the plants in the different series are closely related to the final nodule numbers: similar differences between treatments occur in top and root weight. The number of nodules per g. root weight are given in Table VII below.

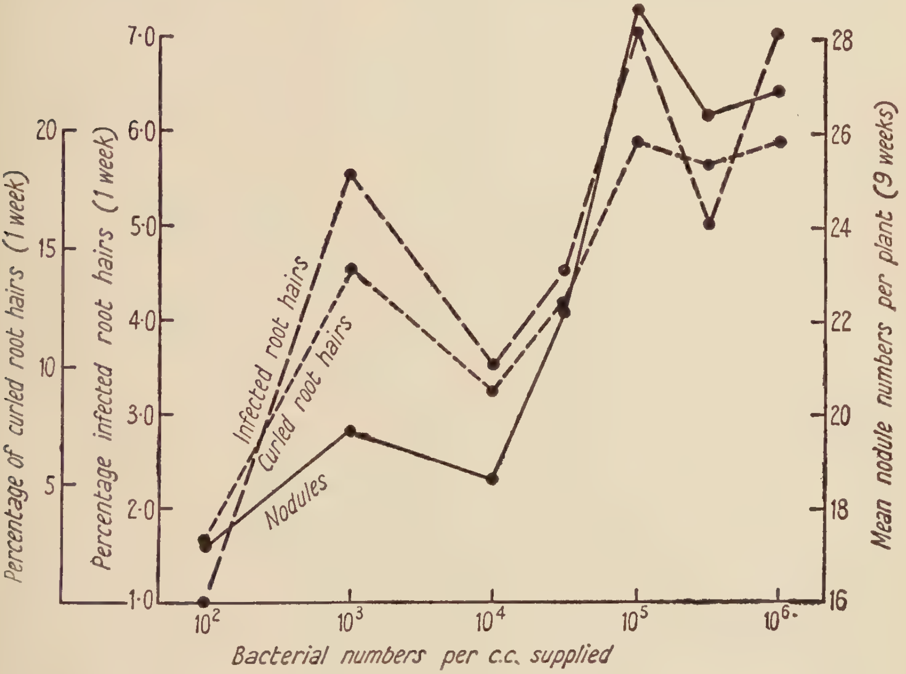

FIG. 2

TABLE VII

Mean Nodules per gramme-root

$$\left(\text{Gramme-root} = \frac{\text{Nodule number} \times 1000}{\text{Dry-weight roots in mg.}}\right)$$

Treatment sets.	Initial bacterial number.	Mean nodules per gramme-root.	n = degrees of freedom.	S.E. of mean from analysis of variance.
A	1×10^6	67·21	9	9·99
B	5×10^5	76·05	9	9·99
C	1×10^5	91·71	9	9·99
D	5×10^4	70·03	8	9·48
E	1×10^4	60·63	9	9·99
F	1×10^3	69·10	9	9·99
G	1×10^2	64·33	9	9·99
Mean		71·31	68	3·16

It is apparent that the differing numbers of nodules between treatments must have been due to the differing sizes of the root systems. The graph in Fig. 1 shows that these divergences in nodule numbers become progressively greater as the plants develop, and it can only be surmised that the same must be true of the root systems. Within a week of infection both nodule number and percentage of infected root-hairs differ between treatments; the denser the bacterial population the larger the nodule number. Owing to the fact that the plants were growing in a medium devoid of nitrogen, the growth of the plants must have become progressively more dependent upon nitrogen fixed in the nodules. Any initial advantage due to a larger number of nodules must therefore have been maintained since growth is dependent upon nitrogen supply and is exponential.

The experiment thus shows that the percentage of infections is related to the number of bacteria in the culture, but that it increases very little with bacterial density (Table VI). The effects of bacterial density on nodule numbers are even less pronounced; thus a thousand-fold increase in bacterial numbers leads only to 60 per cent. increase in final nodule numbers (Table II). Nevertheless, the difference in nodule numbers is established within a week from inoculation, and is later maintained. This cumulative effect is due to the activity of the nodules which result in a greater growth of both top and root in the sets with higher nodule numbers at the start. Since the number of nodules per gramme of root remains constant, nodule number increases with size of root system and hence the final numbers of nodules are related to the numbers developed on the young seedlings, which numbers are themselves related to the numbers of infected root-hairs and thus ultimately to the density of bacterial population of the rhizosphere, of which the percentage of curled root-hairs may be taken as some measure.

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The Dynamics of Cell Expansion by Turgor

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Abstract

It is pointed out that in dynamic cell-states, when alteration in volume is taking place, the turgor pressure and wall pressure need not be equal: it is the difference between them which is the cause of the volume change.

THODAY (1950) has recently criticized Burström (1948) for suggesting that during expansion or contraction of a cell by turgor changes the turgor pressure exerted outwards by the cell contents would not be at all times exactly equal and opposite to the wall pressure exerted inwards by the stretched wall. Whatever views one may hold on the desirability or otherwise of Burström's proposed modifications in the terminology, there can be no doubt whatever that in this statement as applied to the dynamic states of cells in the course of swelling or contracting Burström is perfectly correct.

The statement that action and reaction are equal and opposite is only applicable to, and necessarily true of, static states. During expansion or contraction of a cell, action and reaction need not be equal, and if the expansion or contraction is the result of turgor change they cannot be. If the cell expanded as a whole as a result of an imbibitional change affecting contents and wall exactly alike, they could conceivably remain equal, but this is not the case considered. During expansion by increase of turgor, the turgor pressure must exceed the wall pressure, and during contraction by loss of turgor, the wall pressure must exceed the turgor pressure. It is, in fact, only the difference between the inwardly and outwardly directed pressures which brings about any change in volume as a result of changes in turgor.

The same argument is obviously true of all semi-analogous cases such as the inflation of tyres and footballs and so on. While air is not being pumped in, that is to say during purely static states, the action and reaction are, of course, equal and opposite, but during a stroke of the pump the air pressure exceeds the inwardly directed reaction of the tyre or football and the excess pressure outwards causes the further stretching. Stretching and expansion take place to the point at which the higher tension in the more expanded tyre or football is just sufficient to set up an inwardly directed pressure equivalent to the slightly greater internal pressure at the end of the stroke. If the opposite forces remained at all times exactly equal, as maintained by

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Thoday, there would be no resultant force in either direction and nothing in the case of the cell to make the cell either expand or contract.

On Thoday's contention no contraction or expansion by turgor could ever take place. The difference between the action and reaction is in fact the moving force bringing about volume changes and together with the resistance to movement of water into or out of the cell decides the rate of expansion or contraction. Resistances and rates of change in volume may admittedly be very small and the difference between the opposing pressures therefore also small, but if such a difference did not exist at all there would be no explanation of change in volume by turgor changes. If the turgor pressure never exceeds the wall pressure, what makes the cell expand?

It is admitted that the realization of the difference between turgor pressure and wall pressure in dynamic states, during changes in volume, may seldom be of practical importance and that except during rapid volume changes a measurement of one might be taken as a sufficiently good approximation in estimating the other. But to deny the possibility of the difference between the action and reaction during changes in volume is to take up a dynamically impossible attitude and to shut one's eyes to the moving force responsible for all the volume changes under consideration.

LITERATURE CITED

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Observations on the Biology of *Fomes annosus*, with Particular Reference to East Anglian Pine Plantations

III. Natural and Experimental Infection of Pines, and Some Factors affecting Severity of the Disease

BY

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With Plate XII and two Figures in the Text

ABSTRACT

Apparently *Fomes annosus* normally invades healthy, not damaged, roots, the wood being infected almost at once in small roots, though in larger ones the fungus grows in the bark for some time. Alkaline soils also favour the latter type of development. Infections of living roots are more frequent around infected stumps in alkaline soils than in acid ones.

Details are given of infection of pine roots induced experimentally and of the factors affecting invasion.

Greater resistance to killing is evinced by older trees and those on the margin of the plantation. Disease is less severe in East Anglia on acid soils and where there is more organic matter on the soil surface. These relations are apparently associated with the types of soil microflora (of which details are given), the antagonism of *Trichoderme viride* being especially important.

NATURAL INFECTION OF TREES

i. *Invasion of the living root*

FOMES ANNOSUS Fr. first appears on living pine roots at the points of contact with roots already infected by the fungus. Prior to invasion, living roots seem outwardly healthy and their internal structure is also normal. No clear instance was found where invasion had been preceded by the formation of abnormal zones in the root, as described by Day (1946). *Hylastes ater* Payk. occasionally makes tunnels in living roots around stumps, but such roots are not more likely to be invaded by *F. annosus* than normal ones. Similarly, there is no evidence that dead roots afford a means of entry for the fungus (Rishbeth, 1950).

The following observations were made in plantations 15–25 years old in which trees were attacked by *F. annosus*. The mycelium passes directly from the bark of infected roots on to that of living roots and often grows superficially along the surface before penetrating living tissues. The extent of

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superficial growth varies with the soil type. In acid soils, formerly bearing woodland or *Calluna* heath, it occurs only on pine roots lying at a depth of 30 cm. and below; moreover, such growth is relatively feeble. In alkaline soils, however, *F. annosus* often grows superficially on roots at all depths. In a plantation on former arable, for instance, it was found on the outer bark scales up to a metre in advance of the point of invasion into living tissues. As pointed out in an earlier paper (Rishbeth, 1950), this ability of the parasite to grow along root surfaces is associated with a relative scarcity of competing fungi in the bark scales. The following experiment provides further evidence of the varying extent to which *F. annosus* grows on the surface of roots obtained from different types of site. Inocula consisting of short lengths of root permeated by *F. annosus* were placed on samples of unsterilized roots (diameter 0·5–1·0 cm.) taken from a depth of about 20 cm., and incubated under the conditions described in the paper already quoted. The extent of growth of *F. annosus* from inocula on to the roots was classified as follows: 0, none; 1, slight; 2, moderate; 3, abundant. The results are given in Table I.

TABLE I

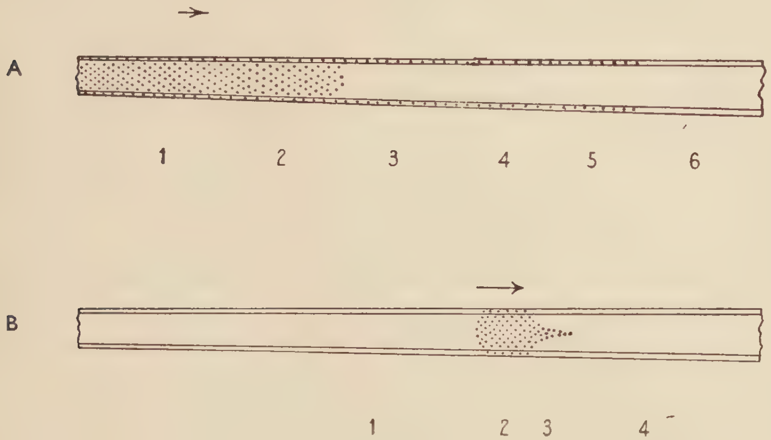
Growth of F. annosus *on Unsterilized Pine Roots*

Former status of site.	No. of sites.	No. of root samples.	No. of samples giving growth from the inoculum in the groups.				Average relative growth per root.
			0	1	2	3	
Woodland (acid)	7	38	22	14	1	1	0·5
Woodland (alk.)	5	26	2	7	8	9	1·9
Heath (acid)	8	46	38	3	5	0	0·3
Heath (alk.)	5	28	2	5	6	15	2·2
Arable (alk.)	8	44	0	9	13	22	2·3

The abundant growth of *F. annosus* on root samples from alkaline soils is in striking contrast to its poor growth on those from acid soils. In the former soils the parasite grows along the surface of roots, presumably with little opposition from other fungi, and enters the bark at various places. As a result, small branch roots are often killed and the fungus sometimes invades the wood well in advance of the region where the main penetration has occurred. It is possible that this form of attack lowers the resistance of roots to further invasion. No such by-passing action takes place on roots in most acid soils investigated. Here, owing to competition with fungi on the root surface, *F. annosus* is more strictly confined to living tissues and hence, presumably, encounters a somewhat greater degree of resistance.

F. annosus is apparently able to invade living root tissues at any point, and early stages of invasion are normally marked by a brown discoloration. In small roots, up to about 0·3 cm. in diameter, the wood is often entered soon after the bark. Death of the distal end, as shown by discoloration, looser attachment of the bark to the wood, and rapid invasion by saprophytes then occur almost at once. During its invasion of the living portion of the root the parasite grows in all tissues at about the same rate. Small amounts of resin are commonly exuded during this process.

In roots more than 0·3 cm. diameter, *F. annosus* colonizes the bark to a greater extent before penetrating the wood. Later stages of invasion vary considerably, and the condition found in alkaline soils, of which the root diagrammatically represented in Text-fig. 1, A, is an example, will be considered first. *F. annosus* is present in the living bark well ahead of its penetration into the wood (regions 3 and 4) and occurs superficially on bark scales farther forward still (region 5). The abundant growth of *F. annosus* in the portion of root invaded earlier (region 1) is associated with its slow replacement by other fungi; in such roots the parasite is often still present a metre or more behind the point of farthest penetration. In alkaline soils considerable lengths



TEXT-FIG. 1. Diagrams showing invasion of pine roots by *F. annosus* (× ½).

A. *Infected root from alkaline soil.*

Region.	Extent (cm.).	Characteristics of region.
1	>15	Little resin exudation; wood dry; *F. annosus* hyphae abundant.
2	10	Marked resin exudation; wood resinous; hyphae less abundant.
3	10	Marked resin exudation; wood resinous; hyphae in bark only.
4	17	Local resin exudation; wood not resinous; hyphae in bark only.
5	25	Bark scales whitish, peeling; a few superficial hyphae present.
6	—	Root normal, bark scales intact.

B. *Infected root from acid soil.*

1	>15	Bark split by resin; wood resinous; secondary fungi present only.
2	4	Marked resin exudation; wood resinous; *F. annosus* hyphae in bark and wood.
3	2	Slight resin exudation; wood resinous; hyphae in centre of wood only.
4	—	Root normal.

▨	Indicates region where *F. annosus* is present.
→	Indicates direction of invasion by *F. annosus*.

of infected pine roots show no resin exudation, and in many instances the wood is scarcely resinous although permeated by *F. annosus*.

A different condition exists in many acid soils, and a root of comparable age from a plantation on former *Calluna* heath is represented diagrammatically in Text-fig. 1, B. *F. annosus* is invading the bark and wood at about the same rate, the farthest point to which the fungus has penetrated being towards the centre of the wood. No purely superficial growth is present. The absence of *F. annosus* in region 1 is due to the rapid replacement by other fungi characteristic of roots in such soils. In further contrast to roots in alkaline soils, wood invaded by the fungus is invariably resin-soaked, and even diminutive roots produce a copious resin exudation.

As invasion progresses, *F. annosus* enters branch roots and eventually kills them. Unless checked, the fungus reaches the bole, where it invades the bark and passes outwards along other main roots. At this stage symptoms usually appear in the crown; death follows after a variable period, depending mainly on the age of the tree. Penetration of lateral roots on the far side of the tree enables the fungus to pass to living roots of adjacent trees, which are in turn invaded. Thus the gap caused by the disease gradually increases in size. Some trees at the edge of such gaps, although not killed outright, are so weakened that they are readily blown over. Wind-throw of *Pinus strobus* affected by *F. annosus* has been recorded by Haasis (1923).

ii. *Variation in the number of infections*

Since the severity of the disease varies considerably from site to site, an attempt was made to discover whether there was any corresponding variation in the number of infections taking place around stumps containing *F. annosus*. Firstly, pine stumps in two contrasting soil types were examined. These stumps had been left by thinning 3 years previously and had been naturally infected by *F. annosus* (Rishbeth, 1951). A search was made for infections on living roots of 25-year-old pines where these touched the stump roots. In connexion with 33 stump roots exposed in an acid soil, formerly *Calluna* heath, 7 infections (0·2 per stump root) were found, whereas with 11 stump roots exposed in an alkaline soil, formerly arable, 8 infections (0·7 per stump root) were discovered.

During investigation of older stumps containing *F. annosus* roots of adjacent trees lying near the stump roots were examined. In a plantation on former arable (alkaline) some 90 per cent. of such roots were dead, whereas around stumps of the same age in a plantation on former *Calluna* heath (acid) only 12 per cent. were dead. Similarly, 30 per cent. dead roots were found near infected stumps in a former woodland site with alkaline soil and 10 per cent. in one with acid soil. These differences probably arose from a greater number of root infections around stumps in the alkaline soils.

Lastly, evidence is available from two plantations of the same age (7 years) which were replanted after fire. The young pines were infected from stumps of the previous crop, which were comparable in size since the fire occurred when each original plantation was 13 years old. In one site, formerly grassland (alkaline), 18 per cent. of the stumps were infected by *F. annosus* and 17 per cent. of the new trees were dead. In the other, formerly *Calluna* heath (acid), 33 per cent. of the stumps contained the fungus but only 10 per cent. of the new trees had been killed. Since other observations suggest that the resistance to disease of trees as young as this varies little with the site, the higher mortality on the alkaline site was almost certainly caused by a larger number of infections from each stump containing *F. annosus*. This hypothesis was eventually proved correct by experimental infections, described later (p. 231).

It has already been suggested that *F. annosus* does not grow freely on roots in many acid soils because of competition with other fungi, and the lower incidence of infections on these roots may partly be explained in this way. The fact that *F. annosus* partially fails to emerge from stump roots in such sites (Rishbeth, 1951), can be regarded as a contributory factor.

iii. *The mode of resistance to invasion*

There is some evidence that invasion of pine roots by *F. annosus* is retarded or checked by resin production. In a typical gap caused by the disease on alkaline soils it is possible to find considerable lengths of root killed by *F. annosus* in which resin production has been very intermittent. It is relatively easy to determine this, since resin decomposes slowly and eventually leaves a black stain. If, furthermore, the gap is becoming stabilized, it is possible to find near the periphery resinous roots from which exudation is considerable. In a number of such gaps an attempt was made to assess the growth rate of *F. annosus* in these two types of root.

For the former type of root the distance to which *F. annosus* had spread from the original source of infection, a stump, along the roots of surrounding trees was measured. Three or four such measurements were obtained in different directions from the stump, and a mean value calculated. Sites were chosen where it was reasonably certain that stumps had been infected by air-borne spores soon after felling. Since the date of felling was known, the annual growth rate along the roots could be estimated. This estimate of the growth rate of the fungus as a parasite was only reliable where the length of stump roots was small in relation to the combined length of the roots of surrounding infected trees. By this method growth rates of 0·6–0·8 m. per year were commonly obtained. For the other type of root with copious resin exudation, infected roots were exposed, marked at the point where resin had most recently exuded, and then covered again with soil. After a year the roots were again exposed and the length along which further resin had been produced was measured. Since the most recent exudation of resin corresponded closely to the position of the fungus in living tissues (Text-fig. 1, A), the growth rate of the fungus could be calculated. In this way, growth rates of 0·2–0·3 m. per year were obtained from the same sites in which the other measurements were made. Taken in conjunction, these results suggest that the rate of invasion of living roots by *F. annosus* is slower when resin production is considerable.

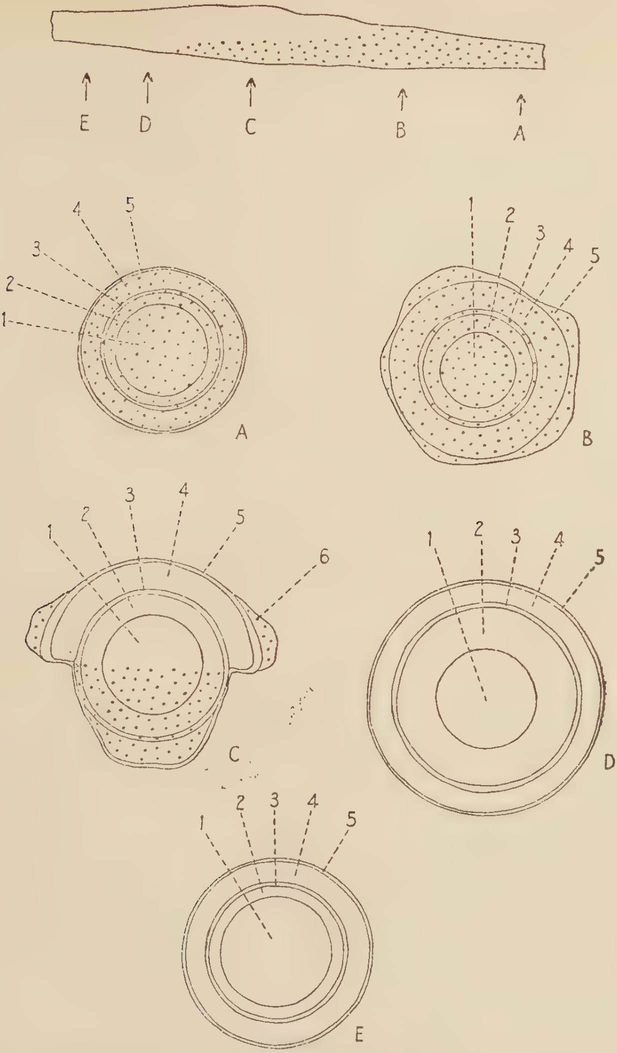
Roots in which growth of *F. annosus* has been checked are invariably resin-soaked; the wood is often hard and flinty, and the annual rings abnormally clear. Abundant resin production is probably the most frequent cause of such cessation of growth of the fungus. Resin production tends to increase with the age of the tree and is rather more vigorous in roots of Corsican pine, *P. nigra* var. *calabrica* Schneid., than in those of Scots pine, *P. sylvestris* L.

F. annosus is also checked to some extent by meristematic activity which, however, occurs only when the parasite is invading roots relatively slowly. Such activity is often found, for instance, at the junction between living and dead tissues in roots near the periphery of almost stabilized disease gaps (Pl. XII, C and D). Here part of the cambium is killed, but the unaffected portion actively proliferates xylem and phloem. Tracheides formed in this way are relatively thick-walled and have a narrow lumen; the sieve tubes are similarly somewhat compact. Isolated areas of meristematic activity are found in the cortex, which is abnormally extensive. The phellogen is active and produces a thick layer of cork; it is often formed well inside the original infected tissues.

The shape of the root in this region varies considerably, and sections reveal that zones of abnormal tissue are disposed in many ways. If only a small amount of cambium is killed, the proliferated tissues arch over it and eventually coalesce so that, after a year or so, a lesion with an elongated scar is the only visible sign of previous infection. If, on the other hand, about half the cambium is killed, the dead and living root portions lie approximately parallel (Text-fig. 2). In the region immediately beyond that invaded by *F. annosus* there is a moderate amount of proliferation, but, since this decreases farther along towards the tree, the root tapers to its normal size in a short distance. The result of experimental infections, described later, suggest that this meristematic activity is a response to fungal invasion.

In some roots apparently recovering from infection, *F. annosus* is present only in the outer tissues which, through the activity of a new phellogen, are sealed off from the living part and eventually disrupted. The formation of a new phellogen is probably the only kind of meristematic activity in infected roots which appreciably restricts the growth of *F. annosus*. Thomas (1934) noted that wound cork is often formed as a reaction to invasion by *Armillaria mellea*. In dead tissues *F. annosus* is often replaced by other fungi. The living portion of the root frequently branches soon after the fungus is halted: in some instances a single large root grows out close to the dead one, but, more often, a cluster of smaller roots is produced (Pl. XII, A).

In former woodland sites on acid soils, living roots around stumps infected with *F. annosus* often bear lesions which probably mark points of former infection by the fungus. It was seldom possible to isolate the parasite from such lesions, although careful search revealed small portions of root containing it. After thinning, a somewhat similar condition is found in sites which were formerly *Calluna* heath, but, in most alkaline sites, lesions associated with



Text-fig. 2. Diagrams of an infected pine root showing meristematic activity: a half-root (× 1), to show the approximate distribution of infected tissue, and transverse sections (× 4), made at the positions indicated.

Regions: 1. Xylem: large, thin-walled cells. 2. Xylem: small, thick-walled cells. 3. Phloem. 4. Cortex. 5. Cork. 6. Disrupted outer tissues.

⁙ Area penetrated by *F. annosus*.

meristematic activity are frequent only in later stages of attack, when the disease is becoming stabilized.

iv. *The relation of disease resistance to other factors*

Rennerfelt (1947) recorded that most pine plantations in south-west Sweden are resistant to attacks by *F. annosus* at an age of 50 years and over.

There is evidence that resistance of pines in East Anglia also increases with age. Trees up to 10 years old on former woodland sites are susceptible to the disease over a wide range of conditions. From 15 to 30 years, however, killing of trees is frequent only on certain types of site, and, above 30 years, it is even less common. The development of disease gaps around infected stumps is of interest in this respect. In many gaps, after a period in which the fungus advances steadily, fewer trees die each year at the periphery and eventually deaths cease altogether. Data from such stabilized gaps in plantations on former arable land are given in Table II. The distance to which the fungus had spread from the stumps was determined by the method already described (p. 225).

TABLE II

Data from Gaps in Plantations at whose Periphery F. annosus *had ceased to advance*

Infected stump: period since felling (years).	Extent of spread of the fungus from the stump (m.).	Approx. growth rate of fungus (m./year).	Approx. age of the trees when the fungus ceased to advance (years).
6	3·5	0·6	25
10	5·5	0·55	23
18	10·6	0·6	24

The stage at which the fungus ceased to advance was clearly independent of the duration of the outbreak, but was related to the age of the trees. Examination of root systems at the margins of recently stabilized disease gaps suggested that the fungus had been checked by increased resistance of individual roots. Although in any one outbreak the fungus is eventually halted, it is unlikely that plantations acquire complete immunity from further attacks, especially if they are on alkaline soils. With more heavy thinning, for instance, the balance may again be shifted in favour of the fungus and more trees may be killed.

Trees at the extreme edge of plantations resist the disease more effectively than those inside. This is clearly seen on alkaline soils where stumps containing *F. annosus* lie just outside plantations and where the stump roots extend to the margin or cross it. The fungus does not kill trees to form a semicircular gap, as might perhaps be expected; several of the marginal trees survive, and an irregular gap is formed behind them. The marginal trees are often stunted, indicating that they have not entirely escaped attack, and examination of the root system reveals that *F. annosus* has been checked at a variable distance from the bole. The probable explanation is that the outer trees receive more light and experience less root competition than the inner ones, and are consequently more resistant. In East Anglia pines are rarely killed by *F. annosus* in narrow shelter-belts, probably because competition between trees is less intense than in plantations and because little felling is done until trees are relatively old and therefore resistant to the disease.

The fact that production of resin by infected roots tends to be greater in acid soils than in alkaline ones suggests that resistance to invasion is correlated with site factors. This relationship is very complex and can more conveniently be discussed when other factors associated with severity of the disease have been considered. It is worth noting here that the resistance offered to the fungus may be influenced by the number of infections sustained by a single root or by the root system as a whole. Leach (1939) made observations which suggest that this is true for *Armillaria mellea*. In East Anglian plantations with alkaline soil the relatively large number of infections occurring on living roots around stumps containing *F. annosus* may lower the resistance of trees to such an extent that root systems are rapidly invaded.

By culturing *F. annosus* on wood blocks Hopffgarten (1933) claimed to show that susceptibility to attack by *F. annosus* of trees growing on former arable was related to the spongy texture and high nitrogen content of the wood. In East Anglia the parasite tends to invade living roots faster in plantations on former arable than on former heath. However, when *F. annosus* grew through the wood of freshly gathered, unsterilized root lengths by the method described in an earlier paper (Rishbeth, 1951), growth rates in roots obtained from the two types of site were not significantly different. This suggests that physical or chemical properties of the wood are not of primary importance in determining resistance of living roots to invasion.

Experimental Infection of Trees

i. *Infection of roots*

Preliminary infection experiments were carried out with 4-year-old Scots pines in large pots containing Breckland soil. Two types of inoculum were used, one consisting of lengths of pine root (average length 8 cm. and diameter 2 cm.) artificially infected with *F. annosus*. Such an inoculum was placed in close contact with the main root. The other type of inoculum was a culture of *F. annosus* in a sawdust medium similar to that used by Badcock (1941); to inoculate roots a small incision was made in the wood by means of a scalpel and sawdust permeated by *F. annosus* mycelium was inserted into it. The incision was then covered with soft grafting wax.

Root systems of a few trees were examined 3 months after inoculation, when one very early stage of infection was found, but the majority of the trees were left for a year. Of 80 young pines inoculated by the first method, only 3 were infected; in these the fungus had invaded the root at the point of contact with the inoculum (Pl. XII, B). None of the 18 trees inoculated by the second method was infected. In this experiment *F. annosus* was rapidly replaced in the pine root inocula by other fungi, and this replacement was probably responsible for the low proportion of roots infected. Competition with other fungi was difficult to avoid: it was found, for instance, that the potted Breckland soil, even if initially free from *Trichoderma viride* Pers. ex Fr., soon developed this fungus under experimental conditions. In view of the low proportion (3 per cent.) of successful root infections obtained, similar inoculations were performed on pines in Breckland.

The plantations chosen varied in age: in four of them the trees were 7–8 years old and in six others they were 15–25 years old. Each of these age groups contained plantations on acid and alkaline soils and each contained one plantation in which some of the trees were Corsican pines. Sites free from stumps were chosen in these plantations. For each tree selected, one lateral root lying at a depth of 15–25 cm. was inoculated, roots nearer the soil surface being avoided since competition from other fungi was generally more intense there. After inoculation by contact with a length of infected root or by insertion of infected sawdust, the roots were re-covered, care being taken to surround them with soil taken from a depth of at least 15 cm. Some roots inoculated by the first method were previously cut to expose the bark and wood at the point of inoculation. Similar roots were inoculated by spraying a suspension of *F. annosus* basidiospores on to the surface; some of these roots were previously injured, as already described. In control roots a scalpel incision into the wood was not inoculated but was covered with wax. Roots were examined a year later for the presence of *F. annosus* and for the presence of lesions associated with meristematic activity of the type already described. The results are given in Table III.

TABLE III

Results obtained by Inoculating in Various Ways the Roots of Pines growing in Plantations

Methods of treatment:

1. Contact with infected inoculum. Root not injured.
2. ,, ,, ,, ,, Root injured.
3. Infected sawdust inserted into root.
4. Inoculated with suspension of basidiospores. Root not injured.
5. ,, ,, ,, ,, ,, ,, Root injured.
6. Control: incision not inoculated.

	Method of treatment.					
	1	2	3	4	5	6
Trees 7–8 years old:						
Total No. trees .	8	6	18	2	5	6
No. with lesions .	5	6	17	1	4	1
No. with *F. annosus* .	2	2	3	0	0	0
Trees 15–25 years old:						
Total No. trees .	13	9	13	8	8	10
No. with lesions .	5	8	10	1	6	3
No. with *F. annosus* .	1	0	1	0	1	0

Formation of lesions was often associated with production of resin, which split the bark at intervals along the root. Tissue was proliferated at the margins of these splits, producing lesions indistinguishable from those formed as a result of natural infections. Lesions present in a proportion of uninoculated roots were presumably formed in response to wounding. It is probable that infection by *F. annosus* also stimulated this response, for a greater proportion of lesions were produced on inoculated than on uninoculated roots, and lesions were formed after treatments 1 and 4 in which roots were not injured before inoculation.

In the single instance where *F. annosus* became established after inoculating with a spore suspension no invasion of the living tissues took place, the fungus being present on the root surface only. With young trees inoculated by *F. annosus* mycelium (methods 1–3), 7 out of 32 roots (22 per cent.) were infected, whereas with older trees similarly inoculated 2 out of 35 (6 per cent.) were infected. This agrees with the previous observation that resistance of trees to *F. annosus* increases with age. Two of the 7 young trees successfully infected were Corsican pines, as were all 3 of the older ones so infected. Although successful infections were too few to make a satisfactory comparison of their incidence in the various soils, it is interesting to find that, for the younger trees, 5 out of 7 were growing in alkaline soil, as were all 3 of the older trees. This agrees with the incidence of the disease in these soils under natural conditions.

It was notable in this experiment, as in others where root inocula of *F. annosus* were used, that roots were found to be infected only where the fungus was still present in the inoculum at the time of examination. Successful infection probably depends upon vigorous and sustained growth of *F. annosus* from the inoculum, but if this fungus is being replaced by others, a point is reached when the food-base is insufficient to allow such growth.

In a further experiment healthy roots were inoculated by placing large pieces of naturally infected root in contact with them. A year later 45 per cent. of infections were found on roots of pines 6–7 years old, but only 15 per cent. on roots of similar size of pines 16–20 years old. Furthermore, in roots of the younger trees *F. annosus* had grown an average distance of 22 cm. in the bark and 13 cm. in the wood, whereas in roots of the older trees it had grown 7 cm. in the bark and had failed to penetrate the wood. Here tree age clearly influenced the resistance of roots to growth of the parasite.

The relative resistance to infection of roots which were normally healthy, severed, or in a weak condition was investigated in four plantations in which the trees were 16–20 years old. Vigorously growing trees provided the first two types of root; some roots were inoculated but otherwise left intact, and others were sawn through about 0·5 m. from the bole, the severed portion being inoculated near the cut. Trees which were heavily suppressed but still living provided the third type of root. Inoculations were made in scalpel incisions with a sawdust culture of *F. annosus.* The results, obtained a year later, are given in Table IV.

Although the number of roots inoculated was relatively small, the differences are striking. The healthy roots had the lowest proportion of infections and in them, as before, the parasite was confined to the bark. The somewhat weakened roots of the suppressed trees were more frequently infected, with some growth of the fungus in the wood. The severed roots were all infected, with extensive growth in the wood. The portion of root still attached to the tree was sometimes infected from the severed portion, where the two were touching. Here, however, the fungus was virtually limited to the bark, which indicates that an attached root, even if cut through near the bole, still retains considerable resistance to invasion. These results will be discussed later.

TABLE IV

A Comparison of the Resistance to Infection of Healthy, Severed, and Weakened Pine Roots

Condition of tree.	Total No.	Roots inoculated. No. with *F. annosus*.	% with *F. annosus*.	Length of root invaded (cm.).*
Healthy, root uncut	14	2	14	0
,, ,, sawn through	11	11	100	80
Suppressed, root uncut	9	3	33	30

* Represents the average distance which *F. annosus* had grown in the wood distally from the point of inoculation.

Since the affected areas in East Anglia have a low rainfall and very permeable soils, an attempt was made to discover whether the resistance of pines to *F. annosus* was related to the amount of water available. To facilitate control of water-supply the experiment was carried out with 4-year-old Scots pines in pots containing Breckland soil. The young trees were planted in such a way that most of the small branch roots touched a large inoculum containing *F. annosus*. Three sets of twenty pots each were prepared. Set 1 was watered every day during the summer, when they were in the open, and every other day during the rest of the year. They were put under cover during the winter only. Set 2 was lightly watered every week during the summer and every other week during the rest of the year; although well illuminated, they remained under cover throughout the year. Owing to the light watering and evaporation from pots, the soil was consistently drier in this set than in set 3, which was sunk in a well-drained sand pit and received only rainfall throughout the year.

The root systems were examined after 18 months. For each tree a note was made of the number of infected and uninfected roots exceeding 1 mm. diameter. For infected roots the length killed by *F. annosus* was recorded, as was the presence of any meristematic activity. Data obtained from root systems of infected trees are given in Table V.

Sets 1 and 3 provide the best comparison, owing to the greater number of trees infected. The mean number of infected roots per tree is the same in the two sets, whereas the mean number of uninfected roots was greater in set 1 (the difference was significant). The total length of roots killed by *F. annosus* was significantly greater in set 3. Meristematic activity was sporadic in infected roots of set 1, but absent in set 3. These differences suggest a somewhat greater root activity and resistance to invasion in set 1. The meristematic activity recorded in set 1 resulted in the formation of new tissues and root branches, as described earlier (p. 226); in the plantations such activity was found only in much older trees. The high proportion of infected roots present in infected trees of set 2 suggests that restricted watering induced a low resistance to *F. annosus*. The average dry weight of the root system of uninfected trees in the sets was as follows: set 1, 10·9 g.; set 2, 2·5 g.; set 3, 5·1 g. The conditions of water-supply in set 3 most closely resembled those occurring in young plantations, and it is interesting to find that root growth in this set, as shown by dry weight, was intermediate between that in the other two. An adequate water-supply thus probably increases the resistance of young pine roots to *F. annosus*.

TABLE V

Growth of Scots Pines in Pots under Various Conditions of Water-supply: Data obtained from Infected Root Systems

Treatment (and No. of trees infected).	Mean values per infected tree. No. of infected roots.	No. of uninfected roots.	Total length of roots killed by *F. annosus* (cm.).	No. of infected roots showing meristematic activity.
Set 1. Heavy watering (6)	4	12	20	2
Set 2. Restricted watering (2)	5	1	50	0
Set 3. Rainfall only (5)	4	5	50	0

The three factors so far considered, namely, tree age, root vigour, and water-supply, presumably operate by affecting host resistance. Another factor which might well influence severity of the disease is the number of roots infected. Field observations already quoted (p. 224) suggest that, around stumps containing *F. annosus*, more living roots are infected in alkaline soils than in acid ones. The following experiment was set up to show whether this is so. Thirty 4-year-old Scots pines were planted in each of these two contrasting soils, their roots being arranged in such a way that the majority touched a large root inoculum containing *F. annosus*. After a year the number of roots infected by *F. annosus* was counted. The distribution of the parasite showed clearly that the number of infected roots could be taken as roughly equivalent to the number of initial infections. The results obtained are given in Table VI.

TABLE VI

The Number of Infections which occurred on Young Pine Roots touching Inocula of F. annosus; *Results from Two Contrasting Soils*

Soil type.	No. of inocula (out of 30) still containing *F. annosus*.	No. of trees infected.	Total No. of roots infected.
Acid, bearing *Calluna*	17	10	55
Alkaline, former arable	20	17	131

Here, as before, root infections were found only where the inoculum still contained *F. annosus* at the time of examination and hence, for the purposes of calculation, such inocula alone are counted as potentially infective. The average bulk of inoculum in each soil was similar. The two means, obtained by dividing the total number of infected roots by the number of potentially infective inocula, are significantly different, and therefore the difference in the number of infections taking place in the two soils is real.

ii. *Infection of stems and branches*

Since young pines are occasionally damaged at the base of the stem by weevils or rabbits, it is of interest to know whether *F. annosus* can colonize wounds in this position. Four-year-old Scots pines were inoculated after a small incision had been made with a scalpel in the base of the stem. With one method of inoculation a suspension of basidiospores was sprayed into the incision, which was then covered with soft grafting wax. Of 28 trees so inoculated, none became infected. With another method a sawdust culture of *F. annosus* was inserted into the incision, which was then covered as before. Of 36 trees thus inoculated, none were infected. In each experiment about half the trees were in pots and the remainder in Breckland. From the results it seems unlikely that *F. annosus* colonizes wounds of this kind under natural conditions.

The ability of *F. annosus* to infect branches of 7-year-old Scots pines by means of basidiospores was also investigated. Freshly cut branch-ends (of average diameter 0·8 cm.) were inoculated with a spore suspension and then protected from drying by covering with a moist sterile test-tube. Inoculated branch-ends were removed from the trees at intervals and examined for the presence of *F. annosus*; the results are given in Table VII.

TABLE VII

Results obtained by Inoculating the Cut End of Young Pine Branches with a Suspension of F. annosus *Basidiospores*

Period after inoculation.	No. of branches.	No. with *F. annosus*.	Penetration (cm.).*
6 weeks	10	10	0·2 (9), 1·0 (1)
5 months	5	2	1–2
8 months	5	0	—
Not inoculated (control)	10	0	—

* The depth to which *F. annosus* penetrated the wood below the cut surface.

By 8 months after inoculation *F. annosus* was entirely replaced by other fungi, including some causing 'blue-stain', and therefore the farthest extent of its penetration was not determined. It is clear, however, that the parasite showed a very limited power of invasion under these conditions.

In the plantations *F. annosus* is rarely found in roots of pines killed by suppression, but it was discovered in one such tree 2 years after brashing, which entails removal of suppressed branches. This tree was evidently alive at the time of brashing, and so it was possible that the parasite had entered the weakened stem through wounds and had grown down to the base. An experiment to test this possibility was carried out in a plantation of 21-year-old pines. To simulate brashing wounds stems were cut about 2 metres above the ground to expose the wood; the wounds were then inoculated with a suspension of basidiospores. Two kinds of tree were chosen, some being markedly suppressed but still living and others of comparable girth growing vigorously. Some of the suppressed trees were wounded but not inoculated, and acted as controls. These three groups of trees, each 12 in number, were felled after 7 months. A sample of stem, including about 20 cm. on either side of the wounded region, was tested for the presence of *F. annosus*. The results are given in Table VIII.

TABLE VIII

A Comparison of the Resistance to Infection of Suppressed and Vigorous Pines after Inoculation of stem wounds with F. annosus *basidiospores*

		Condition of tree at end of experiment.			
		Dead.		Alive.	
		F. annosus:			
Condition of tree at beginning of experiment.	Treatment.	Present.	Absent.	Present.	Absent.
Suppressed	Inoculated	5	3	0	4
,,	Not inoculated	0	10	0	2
Vigorous	Inoculated	0	0	0	12

Of the 8 suppressed trees which were inoculated and died during the experiment, 5 were infected by *F. annosus*. None of the 4 similar trees which remained alive were infected, neither were the vigorous trees, all of which remained alive. Unless a pine is so weakened as to be almost at the point of death infection of stem wounds by *F. annosus* spores is unlikely to occur.

iii. *Discussion*

The experiments show that infection of roots by contact with a suitable inoculum is achieved at least as readily as by direct inoculation of the fungus into them. Moreover, infection is not dependent on wounding. This agrees with the observation that natural infection of uninjured living roots is brought about frequently, if not invariably, by contact with roots already infected. Under experimental conditions failure to infect roots was often due to replacement of *F. annosus* in inocula by other fungi, but sometimes occurred in the absence of such replacement. This is hardly surprising since under natural conditions some living roots in contact with infected ones fail to develop the disease.

In the laboratory *F. annosus* seldom grew on the surface of unsterilized roots inoculated with a suspension of basidiospores, although growth was more certain if they were cut to expose the bark and wood (Rishbeth, 1950).

In the plantations no infections were obtained by inoculating living roots with spores, although a superficial growth of the fungus was obtained in one instance. The fact that *F. annosus* is rarely, if ever, present in living pine roots in areas lacking infected stumps suggests that spores are negligible as a source of direct infection to roots. Although living roots are occasionally damaged by *Hylastes ater* and by burrowing mammals, there is no indication that wounds thus caused are colonized by spores of *F. annosus*. The probable explanation is that the mycelium produced by a spore has a food-base so small that it is unable to overcome the resistance of living tissues and cause infection. Peace (1938) records that several young trees attacked by the fungus were situated in a line over a vole tunnel. It is unlikely that infection took place in this instance from spores carried by voles; a large, infected stump root running parallel to the tunnel could have produced the same result.

The relatively low resistance to invasion by *F. annosus* of roots of suppressed trees, as compared with those of healthy ones (Table IV), was probably associated with an inadequate food-supply. Similarly, the complete lack of resistance of the severed portion of roots which had been sawn through was doubtless related to loss of food-supply; it contrasted with the considerable resistance of the attached portion of such roots, which still received food. This experiment is similar to one performed by Leach (1939) on roots of *Bridelia micrantha* during study of *Armillaria mellea*; those roots, however, already had localized lesions containing the fungus. *A. mellea* also grew freely in severed roots. Leach pointed out that parasites dormant in root-lesions may become active when trees are felled, and this suggestion is undoubtedly relevant to the case of *F. annosus*.

As briefly indicated in an earlier paper (Rishbeth, 1951), *F. annosus* sometimes enters stumps through roots partially infected at the time of felling. Thus in a plantation containing almost stabilized disease gaps, which had been thinned again, 7 per cent. of the recently cut stumps remote from the disease gaps contained *F. annosus*, whereas 41 per cent. of similar stumps immediately surrounding the gaps were infected. The distribution of *F. annosus* in many of the latter stumps indicated that they had been infected on the side towards the disease gap. This mode of infection, by which other parasites were thought by Napper (1934) to enter jungle stumps, occurs in East Anglian sites only where diseased trees already exist. It is at present unimportant by comparison with stump infection by air-borne spores, but may well become more widespread after further thinning or clear-felling. Since *F. annosus* is replaced by other fungi in dead roots more rapidly in acid than in alkaline soils (Rishbeth, 1951), this mode of stump infection tends to be more prevalent in the latter soils.

The fact mentioned above, that roots of suppressed trees are relatively susceptible to invasion by *F. annosus*, is of further interest. Just before death of the tree it is likely that the resistance of such roots is even lower and that they would be infected by *F. annosus* if it were present in the soil. Since *F. annosus* is rare in roots of trees killed by suppression (Rishbeth, 1950), it is unlikely that the fungus is widely present in soil. Thus it is improbable that roots weakened in other ways, for instance by adverse soil conditions, are infected from the soil, as some investigators have suggested. Such roots are almost certainly infected by contact with others already containing *F. annosus*, except in rare instances where the parasite grows down into them from stem wounds.

Although experiments show that resistance to infection increases with age of the tree, they do not indicate how such resistance is related to the age of individual roots. Field observations suggest that up to about 10 years of age, pines show little resistance in any part of the root system, but that above this age they often show marked resistance in roots only 3 years old. Hence the age of the individual root is probably a less important factor in determining resistance than the age of the tree itself.

The failure to infect wounds in the stem base of young trees with basidiospores is consistent with the observation that moderately severe wounding in this region by weevils or by rabbits does not lead to infection by *F. annosus*. Similarly the results obtained with older trees agree with the observation that vigorous pines are never infected by *F. annosus* as a result of operations such as brashing. Recent work on *Tsuga heterophylla* by Rhoads and Wright (1946), however, suggests that this is not necessarily true of all conifers. The transfer of *F. annosus* to an unwounded stem by contact with an infected one just above soil level, as described by Day (1948), must be relatively rare. The success of such an infection might well have been due to a previous weakening of the tree, since the roots would in all probability have been heavily infected. The experimental infection by spores of greatly weakened trees through stem wounds offers a possible explanation of the appearance of *F. annosus* in a plantation following severe damage by deer, as was observed in King's Forest, Suffolk.

SOME FACTORS AFFECTING SEVERITY OF THE DISEASE

i. *Introduction*

The extent of the damage caused by *F. annosus* in East Anglian plantations is determined by a number of interacting factors. Sites planted for the first time are free from disease until trees are cut down for any reason. After cutting, the number of stumps which become infected with the parasite partially determines the extent of subsequent damage. For instance, a heavy thinning, producing a large number of stumps, will tend to be followed by a worse attack than a light thinning. Again, the proportion of freshly cut stumps infected by *F. annosus* fluctuates throughout the year, and therefore the season at which trees are cut down indirectly affects the incidence of the disease. The age at which the trees are first cut is yet another factor, for resistance of trees to the disease increases markedly between 15 and 25 years. The importance of these last two factors can be clearly seen from the results of early thinning carried out in plantations near Swaffham, Norfolk. Thinning was relatively light, but the proportion of stumps infected was high and the trees were only 14 years old at the time: a severe attack followed, in which some 15–20 per cent. of the trees were killed. Under similar conditions in Rendlesham Forest, east Suffolk, a somewhat heavier thinning led in some instances to attacks where nearly half the trees were killed.

Over and above these factors, the type of site has an important influence on severity of attack. For instance, in a plantation on former *Calluna* heath, thinned at an age of 14 years, an average of 2 trees were killed near each stump infected by *F. annosus*. The parasite invaded root systems at a rate of 0·3 m./year and was checked after about 4 years. In a plantation on former arable, thinned at the same age, about 20 trees were killed around every infected stump, the fungus grew at 0·6 m./year in the root systems, and gaps only ceased to enlarge after about 9 years. The following section is concerned with some environmental factors associated with such outbreaks of varied severity.

The most satisfactory estimates of severity of the disease were obtained from former woodland sites because the frequency of stumps was similar throughout and trees were subject to infection at the same age, soon after planting. Two rectangular plots, each containing 150 trees, were chosen at random in a plantation and the number of trees killed by *F. annosus* was counted. If the estimates differed greatly, counts were made in two more plots. For each plantation the percentage of trees killed by the fungus was calculated. To eliminate differences arising, for example, from varied age of plantations at the time of counting, such estimates were made in a number of plantations on each type of site. Some applications of this method are given later (pp. 239–40).

ii. *Physical factors*

Waterlogging and drought are considered by Day (1946) to be important factors predisposing pines to attack by *F. annosus* in East Anglia. The former will be considered first. Some indication of the depth of the water-table at various situations in Breckland can be obtained from well records. Although there is seasonal variation in level, it appears that the water-table is likely to rise within 3 or 4 metres of the soil surface only in relatively few plantations, situated in the river valleys and at the border of the fens. The water-table is lower than this in most plantations and, in considerable areas between the valleys, it is over 30 metres below soil surface. Thus, in most plantations, waterlogging is unlikely to follow a rise of water-table; it has only once been observed in the forest area as far as is known. Although the courses by which water drains down through the soil after rain are irregularly distributed, there is no evidence of serious impedance. In Breckland, therefore, damage to root systems through waterlogging is likely to be rare, and the distribution of diseased plantations in fact suggests no correlation with this factor.

On the other hand, recurrent droughts are characteristic of East Anglia. The sandy soils often have a saturation capacity as low as 30 per cent., although this value increases with the proportion of chalky boulder clay.

The moisture content of surface soil (0–20 cm.) is 10 per cent. or more after rain showers, but values of 5 per cent. are common even after a wet season. During drought the soil often dries out to a moisture content of about 2 per cent. from the surface down to a depth of at least a metre. Despite this, visible signs of water deficiency in trees are very rare and no instance was seen where abnormal zones in roots could be clearly related to such a deficiency. It is possible, however, that resistance of trees to disease is lowered by periods of water shortage.

In the plantations the amount of organic matter at the soil surface varies; where it is abundant, the surface layer remains moist even during exceptional drought. Since roots are abundant in this layer, it is conceivable that trees in such situations do not suffer a severe shortage of water. An attempt was therefore made to discover whether there was any correlation between amount of surface organic matter and severity of the disease. Standard samples 3 cm. thick were taken from organic matter and soil directly underlying the surface litter. Such samples were collected in all plantations where the disease was evident and also in others where infected roots were found, although few or no trees had been killed. The samples were dried and passed through a sieve of 2-mm. mesh, after which representative sub-samples were ignited. The percentage loss of weight gave a rough measure of the amount of organic matter present. Different types of organic matter were not distinguished since the primary object was to investigate water-retaining capacity. The values so obtained were divided for convenience into three groups. The number of plantations of various types having values which fall into these groups is given in Table IX. Values for severity of the disease on former woodland sites, obtained by the method already described, are also given.

TABLE IX

Diseased Plantations: values of Surface Organic Matter and the Relation to Severity of the Disease

	Surface organic matter, as measured by % loss of weight on ignition.		
	1–10 (group 1).	11–20 (group 2).	>20 (group 3).
A. No. of diseased plantations:			
On former arable	22	0	0
,, heath	9	3	2
,, woodland . . .	16	9	7
B. Severity of the disease:			
The mean % of trees killed on former woodland sites	12	7	2

The relative deficiency in surface organic matter of former arable sites is clearly shown. The similar deficiency of some former woodland sites is probably associated with burning before replanting, since the surface soils contained many charcoal fragments. The mean percentages of trees killed in plantations on former woodland sites comprising groups 1 and 2 are significantly greater than that in similar plantations comprising group 3, and hence severity of the disease is related to the amount of surface organic matter.

Further observations made on surface soil samples showed that the percentage loss of weight on ignition was related to saturation capacity, as might be expected. It was also related to field moisture-content, determined after a period of exceptionally dry weather in the late summer of 1947. It is possible, therefore, that trees growing in plantations where surface organic matter is relatively abundant are resistant to disease partly because they have an adequate water-supply during drought. That resistance to the disease is in fact increased by an adequate water-supply, at least in young trees, is shown by an experiment described earlier (p. 232).

iii. *Chemical factors*

Striking differences in incidence of the disease are sometimes found in adjacent sites where physical conditions of the soil appear to be similar. Analyses were carried out on soil samples taken at a depth of 20 cm. from such pairs of sites. Values obtained for organic carbon, total nitrogen, phosphate, and potash were very similar throughout, but those for pH and calcium carbonate varied considerably; such values tended to be high where outbreaks were severe and low where they were mild. As a result, it was decided to make a survey of soil reaction in all sites where *F. annosus* was causing infection. In order to avoid fluctuations in value which often occurred towards the soil surface, samples were taken at a depth of 20 cm. pH was estimated by means of a glass electrode within 24 hours of collection, a 1 : 5 suspension of soil in distilled water being used for the purpose. Values obtained were divided into three groups, and the number of plantations of various types having values which fall into these groups is given in Table X. Values for severity of the disease are given for two of the groups.

TABLE X

Diseased Plantations: pH of the Soil and the Relation to Severity of the Disease

	pH value.		
	4·0–5·9 (group 1).	6·0–6·9 (group 2).	7·0–8·9 (group 3).
A. No. of diseased plantations:			
On former arable	1	4	16
,, heath	4	3	6
,, woodland . . .	21	2	11
B. Severity of the disease:			
The mean % of trees killed on former woodland sites	4	—	16

Most former arable sites are alkaline. The high pH values of some former heath sites arise from the inclusion, for convenience, of grasslands where chalk is near the surface. The mean percentage of trees killed in plantations on former woodland sites comprising group 3 is significantly greater than that in similar plantations comprising group 1, and severity of disease is consequently related to soil pH.

The values of surface organic matter and pH tend to be associated. Thus in group 3, referred to previously, having the highest value for surface organic matter, all plantations have a low pH. Conversely, in group 1, having the lowest value for surface organic matter, 7 of the 9 plantations on former heath have a high pH. This association may arise as the result of more rapid breakdown of surface organic matter on alkaline soils (Waksman, 1936). Since, however, disease is severe in some plantations where values of surface organic matter and pH are both low, there is good reason for believing that the two factors operate independently.

iv. *Biological factors*

Experiments and field observations suggest that there is a relationship between severity of the disease and the type of soil microflora. An outline study of the microflora was therefore made for a range of soils where the disease was of varied severity. The activity of root-surface organisms had already been investigated to some extent by testing the growth of *F. annosus* on unsterilized roots (Table I). Study of the soil population as a whole, although not so relevant to the activity of *F. annosus* as that of the root-surface population, was expected to throw some light on the distribution of *Trichoderma viride*, one of the fungi antagonistic to *F. annosus* (Rishbeth, 1950). Soil samples were collected in a variety of plantations from depths of 8 and 20 cm. A 1-in-10 suspension of the soil in sterile distilled water and two or three successive tenfold dilutions of it were plated with Dox's agar. Plates were incubated at 22·5° C. for a fortnight, after which the dominant fungi in each series were recorded.

In acid soils of former woodland and *Calluna* heath, where outbreaks of the disease were relatively mild, species of *Penicillium* were abundant. *P. piscarium* Westling was common and *P. janthinellum* Biourge and *P. terlikowskii* Zaleski were often present. In addition, *Mucor ramannianus* Moll., *Zygorrhynchus vuillemini* Namyslowski, and *Trichoderma viride* Pers. ex Fr. were regularly found in such soils. Alkaline soils of a wide range of sites, where outbreaks of disease were more or less severe, had a markedly different microflora. Species of *Penicillium* were much less numerous, *P. janczewskii* Zaleski being the only one frequently present. The genera *Thielavia*, *Stysanus*, and *Phoma* were commonly represented and a few fungi, for example *Gliomastix convoluta* var. *felina* (Marchal) Mason, were more abundant than in acid soils. *Trichoderma viride* was distributed very irregularly and was less frequent than in acid soils.

Counts of micro-organisms were also made for most soil samples. The count of fungi was somewhat greater in acid soils than in alkaline ones, but the position was reversed for bacteria and actinomycetes. The count of micro-organisms was smaller at the lower depth of sampling, as has been noted by other workers.

In addition, the highest dilution at which *Trichoderma viride* developed was noted and its abundance recorded by means of a numerical scale. Thus 0 represented absence of the fungus, 1 its presence at a dilution of 1/10, 2 its presence at a dilution of 1/100, and so forth. Estimates for each sample from a certain type of site, e.g. acid woodland (depth 8 cm.), were added and the total divided by the number of samples: this derivative was called 'average abundance'. Estimates of the abundance of *T. viride* in a variety of soils are given in Table XI.

TABLE XI

Estimates of the Abundance of Trichoderma viride in *Plantation Soils*

			Abundance of *T. viride.*						
				No. of samples in groups.					
Former status of site.	Soil reaction.	Depth of sample (cm.).	Total No. of samples.	0	1	2	3	4	'Average abundance'.
Woodland	Acid	8	22	4	0	7	10	1	2·2
,,	,,	20	21	10	0	3	7	1	1·5
,,	Alk.	8	10	6	0	2	2	0	1·0
,,	,,	20	11	9	1	1	0	0	0·3
Heath	Acid	8	12	2	0	4	5	1	2·3
,,	,,	20	15	3	2	7	3	0	1·7
Grassland	Alk.	8	9	3	2	4	0	0	1·1
,,	,,	20	9	7	1	0	1	0	0·4
Arable	Acid	20	12	7	1	4	0	0	0·8
,,	Alk.	8	10	8	0	2	0	0	0·4
,,	,,	20	12	10	0	2	0	0	0·3

Since values of 'average abundance' for *T. viride* are on a logarithmic scale, the differences which they represent are striking. The fungus is more abundant in acid soils at each depth of sampling. Alkaline soils, formerly arable, differ from other alkaline ones in having less *T. viride* at a depth of 8 cm. Acid soils (pH about 6·0), formerly arable, most of which were situated in Tunstall Forest, east Suffolk, resembled alkaline ones in giving relatively high counts of bacteria and actinomycetes and relatively low counts of fungi. *T. viride* was more abundant at 20 cm. than in corresponding alkaline soils and outbreaks of disease were rather less severe.

These results agree with those obtained from growing *F. annosus* on unsterilized roots (Table I), in that greater growth was obtained for sites where *T. viride* was scarce than for those where it was abundant. The relationship between alkaline soils and relatively severe outbreaks of the disease appears therefore to arise, at least in part, from the type of microflora characteristic of such soils.

V. *Discussion*

It is now possible to draw tentative conclusions about the complex of factors determining severity of attack by *F. annosus* in the pine plantations. It is difficult in practice to distinguish between the effect of factors influencing activity of the fungus and that of factors affecting host resistance, but, for convenience, the two will be considered separately. Some of the former factors are independent of soil conditions and determine the potential inoculum for root infections. Of these, the number of trees felled during thinning, for instance, and the proportion of stumps subsequently infected by *F. annosus* are probably the most important. Others, whose operation depends on the type of microflora, are closely linked with soil conditions. One such factor is the rate of replacement of *F. annosus* by other fungi in stumps, which governs the rate of disappearance of potential root-disease inoculum. This factor is probably not decisive, at least in outbreaks following thinning, because the important initial infections occur within 1 to 3 years of the time of felling, i.e. before the parasite is replaced to any great extent. The ability of *F. annosus* mycelium to break through to the surface of stump roots which it occupies and the readiness with which it becomes established on the surface of living roots are further factors influenced by the type of soil microflora. Since these factors jointly determine the number of infections taking place on living roots, they are of great importance. When *F. annosus* grows on the surface of living roots it often kills small branch roots and invades the wood in advance of the main region of penetration. The extent of such surface growth is largely determined by the soil microflora; thus, in this way also, the latter may influence severity of attack. It is worth emphasizing that, with respect to each of these factors associated with site, *F. annosus* has an advantage in alkaline soils, since there is relatively little competition from other fungi.

The factors associated with host resistance are more obscure, chiefly because fundamental problems of water relations and tree nutrition are involved. It seems reasonably certain that resistance of pines to killing by *F. annosus* increases with age and that such resistance is associated with an increased capacity of the roots to produce resin. Resin production by roots tends to be greater in acid soils than in alkaline ones, and this largely explains the slower growth of *F. annosus* along roots in the former soils. The greater apparent resistance of such roots may in part be due to the relatively few infections sustained, but it probably also reflects greater vigour of the trees. The considerable resistance to the disease of marginal trees in plantations on alkaline soils suggests that the balance between resistance and susceptibility is delicate and also that growth in an alkaline soil does not necessarily lead to low resistance. It is more likely that, on alkaline soils, certain conditions of water-supply or of nutrition favour marginal trees as compared with inner ones, and that these conditions tend to exist more widely in plantations on acid soils.

If one of these conditions is availability of moisture during drought, which is possible in view of the absence of a deep water-supply over most of the forest area, then trees growing on acid soils would be expected to have an advantage since surface organic matter capable of retaining moisture tends to be abundant there. A high pH value and calcium carbonate content might, on the other hand, be associated with an unsatisfactory type of nutrition, which could account for the lower resistance of trees in alkaline soils.

CONCLUSIONS

Living roots invaded by *F. annosus* appear healthy at the time of infection. In small roots the wood is invaded soon after initial infection, but in larger ones growth is extensive in the bark before the fungus penetrates the wood. The progress of invasion differs with the type of site: in alkaline soils the parasite grows freely in the bark ahead of its penetration into the wood, and superficial growth is often extensive. In acid soils, however, bark and wood are invaded at about the same rate and superficial growth on roots is usually absent. More infections on living roots occur around stumps containing *F. annosus* in alkaline soils than in acid ones, probably because of less competition from other fungi.

Invasion of roots by *F. annosus* may be checked by resin production and, to a lesser extent, by meristematic activity. Resistance of pines to killing by *F. annosus* increases with age. Owing to this increased resistance, gaps caused by the disease eventually become stabilized. Trees at the extreme edge of plantations show a greater resistance to the disease than those inside, probably because the former receive more light and experience less root competition. The rate of invasion of roots probably depends more on resistance of living tissues than on physical or chemical properties of the wood.

Pine roots were infected experimentally by placing inocula containing *F. annosus* in contact with them and also by inserting a sawdust culture of the fungus into the wood. Attempts to infect roots by means of basidiospores were unsuccessful. *F. annosus* invaded weakened or severed roots far more readily than normally healthy ones, and roots of younger trees more readily than those of older ones. Roots of young pines were shown to be more resistant to *F. annosus* when watering was heavy than when it was restricted or when trees received rainfall only. Attempts to infect the stem base of young trees were unsuccessful. Cut ends of young branches, inoculated with a suspension of basidiospores, also showed marked resistance to invasion. In plantations, stem wounds were infected by basidiospores only when trees were very weakened by suppression. The relation of these experimental infections to outbreaks of the disease in the plantations is discussed.

Some environmental factors associated with variation in severity of outbreaks are considered, and a method of estimating severity of the disease on former woodland sites is described. Waterlogging of the soil rarely occurs in East Anglian plantations and is thus unlikely to influence severity of the disease. The disease is less severe where there is a relatively large amount of organic matter at the soil surface. Since in these sites the surface layer remains moist during droughts, which frequently occur, it is possible that the trees suffer no great shortage of water and are hence better able to resist the disease.

The disease is more severe where the soil is alkaline. This condition arises naturally where chalk is near the surface and also where the soil has been limed for cultivation. The relation between soil type and severity of the disease arises partly from the type of soil microflora. There are few species of *Penicillium* in alkaline soils and *Trichoderma viride* is not abundant. Both are more frequent in acid soils of former woodland and *Calluna* heath, where outbreaks of the disease are relatively mild; the antagonism of *T. viride* to *F. annosus* is an important factor here.

An attempt is made to assess the relative importance of factors affecting activity of *F. annosus* and host resistance.

Grateful acknowledgement is made to Professor F. T. Brooks, F.R.S., for assistance during the investigation. Thanks are also due to Dr. J. H. Warcup for help in identifying soil fungi and to Dr. K. B. Raper for identification of *Penicillium* species.

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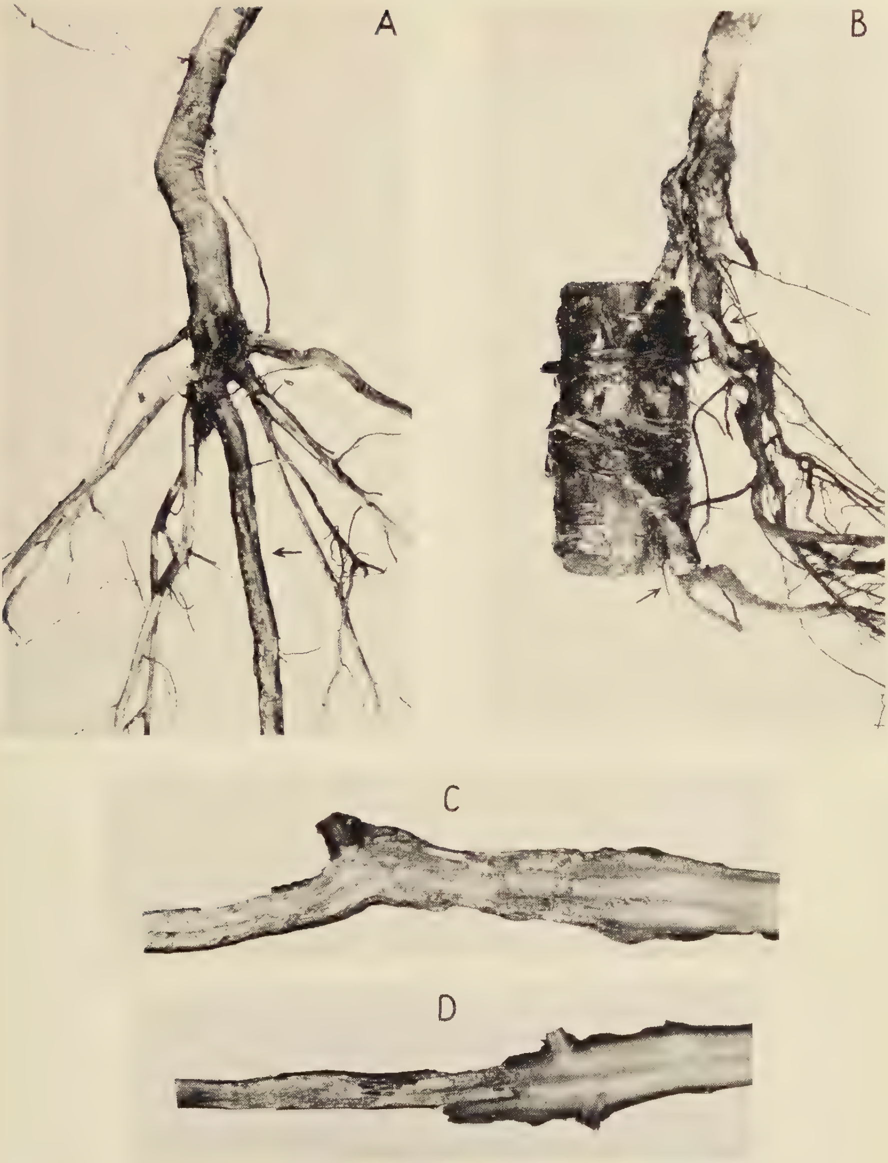
EXPLANATION OF PLATE

A. A pine root in which growth of *F. annosus* has been halted; a cluster of branch roots has arisen soon afterwards. The dead portion (←) still contains *F. annosus*. (× ½.)

B. Root system of a small pine infected during a pot experiment by the inoculum tied to a root. White areas on the roots (←) represent conidia of *F. annosus* which developed during incubation. (× ½.)

C. A halved pine-root, showing a stage where *F. annosus* has been almost checked by resin production. Meristematic activity, as shown by a slight swelling, has recently started in the healthy region to the right. (× 1.)

D. Another halved root in which the fungus has been checked and meristematic activity has continued for several seasons. The base of two newly formed roots can be seen. (× 1.)



J. RISHBETH

The Iron and Manganese Content of Plants present in the Natural Vegetation of the English Lake District

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With six Figures in the Text

ABSTRACT

Estimations of iron and manganese have been made on ninety-six species of plants (including fungi, mosses, ferns, and flowering plants) from a wide range of natural habitats. These plants normally contain more of both elements than is usual in crop plants grown on cultivated fields, probably owing to the greater acidity and humus content of the natural soils. The amount present varies with the individual species, the plant group, and the type of habitat. While some species and groups tend to take up iron or manganese selectively, both the absolute amounts and the ratio of the two elements may vary widely without apparent injury to the plant. In general, these elements accumulate most in aquatic plants growing on organic and anaerobic muds. The lowest amounts are found in species characteristic of flushed brown earths and in plants from drained acid peats. The fruiting bodies of fungi contain little of either element.

INTRODUCTION

IN recent years much attention has been given to the role of minor elements in plant nutrition. However, most of the work on this subject has been devoted to species of agricultural importance, growing in pots or on cultivated soils. Therefore, when an opportunity arose of obtaining vegetation from a wide range of natural habitats, it was felt that analyses for certain of the minor elements might be worth while both intrinsically and for comparison with the data available on crop plants.

Iron and manganese were chosen for study as being likely to show marked differences in amount according to the habitats in which the plants grow. Uptake of these two elements is presumably related, at least in part, to the concentration of soluble forms in the external medium. Thus it might be expected that the availability of iron and manganese would be conditioned by three factors which may vary widely in the soil:

(1) hydrogen-ion concentration,
(2) redox potential,
(3) organic content.

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It is well known that the solubility of iron and manganese compounds generally rises with increasing acidity. Also, anaerobic conditions in the soil have been shown to bring about the reduction of ferric ions and compounds to ferrous forms (Pearsall, 1938; Misra, 1938; Mortimer, 1941). That these are more mobile and able to enter the soil-exchange complex has been demonstrated by Ignatieff (1941) and Gorham (1949). In the presence of organic matter a variety of complex iron humates may be formed. McMurtrey and Robinson (1938) claim that these may remain stable and soluble in conditions of acidity and oxidation at which the element in its ionic state would precipitate as ferric hydroxide. As an example of the influence of organic compounds we may cite the work of Hopkins and Wann (1926), who showed that iron remains available to the alga *Chlorella* in culture solutions at pH 6 only if citrate is added to the medium to prevent its precipitation. In contrast, Bremner et al. (1946) suggest the presence in soils of polyvalent metallo-organic complexes which appear to be non-exchangeable and insoluble in water. These compounds are, however, broken down by organic ions such as citrate and malate, which are known to form co-ordination complexes with polyvalent metals. In all these examples it is probable that manganese and iron may behave in similar fashion.

Another possible influence on the uptake of these elements has been suggested by Olsen (1934). He believes that the concentrations of other elements in the soil may strongly affect manganese uptake. From what is known of mineral metabolism in general, this seems highly probable.

From the above it would seem that a clear correlation of iron or manganese uptake with any one soil factor is rather unlikely. And in fact it is impossible at present to attempt the explanation of many of the results of this survey. Their interpretation must await further knowledge of the chemistry of iron and manganese in the soil as well as of their physiological role in plants.

DATA

The samples were all collected in the southern part of the Lake District, an area of marked relief and high rainfall. The soils are mostly glacial loams and clays deposited over Silurian slates and grits, but plants have also been taken from the occasional limestone outcrops. The habitats sampled may be classed as follows:

I. *Woodland soils*

Calcareous, neutral, and seldom highly organic.

Slightly acid flushed brown earths having a *mull* type of humus mixed with the mineral horizons.

Highly leached podzolic soils, with an acid *mor* humus tending to accumulate on the surface.

II. *Waterlogged soils*

Underwater soils, usually slightly acid, anaerobic, and strongly reducing at rooting levels (Misra, 1938). Ignition loss less than 30 per cent.

Semi-aquatic, ranging from inorganic (silted) marsh soils to highly organic fen and lacustrine peats. Generally moderately reducing, but sometimes oxidized during the summer (Pearsall, 1938), and exhibiting a considerable range of acidity.

Raised bog peats, formed in regions of high humidity by the growth of the bog moss (*Sphagnum*), on waterlogged sites once the level of peat deposition has risen above the influence of ground water. The bogs considered here have all ceased to grow in size or extent, and have been much cut and drained. Drainage has resulted in oxidation of the surface peat, which accounts for its high acidity (Pearsall, 1938*a*). However, reducing conditions probably obtain during much of the growing season, as the peats are still very wet. A further consequence of drainage is the change from *Sphagnum* to *Calluna vulgaris* and *Eriophorum vaginatum* as the dominant species.

It should be emphasized that there is a good deal of intergradation in this classification.

METHODS

Sampling. The plants and the soils in which they were rooted were collected during June, July, and August of 1948 and 1949, an effort being made to sample individuals of the same group of plants at the same time. The woodland trees were all taken in the seedling state.

Leaves were taken except in the case of the fungi, of which the fruiting tops were selected, and the mosses and grassy plants, where the green tops were sampled. All leaves on the plants were collected. The analyses therefore give results for the chief photosynthetic organs, except for the fungi. Only fresh healthy tissues were sampled, from normal plants growing in what appeared (with one or two exceptions as noted) to be their natural habitats. While fresh they were carefully washed with water to exclude surface contamination as far as possible. Afterwards they were placed in a drying-room and stored when air-dry. Three hundred to one thousand milligrams of material were normally used for analysis, but in a few cases of usually solitary species such as *Solidago* as little as 50 mg. were used.

Drying and ashing. Both soils and plants were dried at 100° C. to constant weight and ashed at about 550° C.

Preparation of plant ash for analysis. The ash was extracted with 2 ml. of concentrated hydrochloric acid at 60–70° C. The extract was then diluted to 50 ml. with distilled water and divided into two parts for subsequent analysis.

Iron estimation. 1 ml. of 3N potassium thiocyanate was added to the above extract. The red colour obtained was then compared in a visual colorimeter with a standard iron solution, prepared from reduced iron dissolved in concentrated hydrochloric acid and diluted as required.

Manganese estimation. 1 ml. of phosphoric acid was added to the prepared extract, followed by 0·5–1·0 g. of potassium periodate. The solution was then boiled for 3 minutes, allowed to cool, and made up again to standard volume.

Care had to be taken that the periodate was present in excess, so that the solution did not fade on cooling. Comparison, using a standard prepared from potassium permanganate, was again carried out in the visual colorimeter, phosphoric acid and potassium periodate being added to prevent decomposition during the matching.

pH of the soil. For this determination a glass electrode was used on undiluted soil which had been stored at low temperature in specimen tubes without drying. Tests on fresh and stored soils indicated that the changes on storage were within the normal range of variation for individual samples from the same site during the summer.

While errors are liable to arise from the rather small size of the samples, with consequent difficulty in obtaining homogeneity, the results suggest that these errors lie well within the normal range of variation of the individual species. It is not possible to say how far the results are affected by variation in the age of the plants when collected, or by the differences in the parts sampled from groups such as grass and herbs.

In the presentation of the data, ash is shown as per cent. of dry weight, manganese and iron are given as milligrams per hundred grammes of dry matter (throughout the text referred to simply as milligrams). Soil ignition loss is given as per cent. dry weight.

RESULTS

The analytical results for the individual species are presented in Tables I to IX, in each of which the results are arranged in order of soil pH and with the organic content of the soil added for reference. The tables group the species under the general headings of (I) woodland plants and (II) plants from wet soils. For the woodland species, it has been found convenient to sort out various groups of plants for separate consideration. The plants of wet soils have been grouped differently, according to the three main habitat types. Attention is drawn below to certain features of general interest.

I. *Woodland plants*

Mosses (Table I). This group of plants shows a striking tendency to accumulate iron, the highest figure being 400 mg. Over half the iron values are above the maximum recorded for manganese, which is 77 mg. for a sample of *Plagiothecium elegans* from a conifer wood.

These mosses are representative of three types of habitat. The first three species in Table I were sampled from crevices in impure Coniston limestone and show a high iron content. The next five were on leached mineral soil and show the lowest values for iron among the three groups. The last three specimens were growing on organic *mor* humus accumulations and these also show a high iron uptake, though less than that of the first group. *Dicranum majus*, which was sampled on all three habitats, shows the same picture as the group results. It would appear from this that the impure limestones and the *mor* humus contain relatively more available iron than the leached mineral

TABLE I

Mosses

Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	Soil.	
				pH.	Loss on ignition % dry wt.
Trichostomum tortuosum	11·1	66	400	6·92	—
Hypnum molluscum	8·7	59	204	6·58	—
*Dicranum majus**	3·0	16	166	6·11	35
Catharinea undulata	3·1	10	5	4·70	16
Plagiothecium elegans	6·1	77	30	4·48	19
Mnium hornum	5·7	23	12	4·19	20
Catharinea undulata	4·0	25	69	3·98	25
Dicranum majus	5·3	17	13	3·94	23
Hypnum schreberi	3·2	15	86	3·68	94
Leucobryum glaucum	2·8	8	110	2·93	72
Dicranum majus	3·6	5	160	2·73	95

* From atypical habitat.

soil. Much of the available iron in the last soil has presumably been leached from the surface and is therefore beyond reach of the mosses.

It is of some interest that the two calcicole species, *Trichostomum tortuosum* and *Hypnum molluscum*, have the highest amounts of ash and iron and are also relatively high in manganese.

Ferns (Table II). Among these plants, in contrast to the mosses, there appears to be no selective accumulation of either element to any great extent. The upper level for iron is 53 mg. and the manganese content is usually below 42 mg.

TABLE II

Ferns

Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	Soil.	
				pH.	Loss on ignition % dry wt.
Phyllitis scolopendrium	12·0	18	20	7·05	—
Gymnocarpium robertianum	8·8	20	20	6·70	—
Dryopteris filix-mas	9·4	13	39	5·45	18
Athyrium filix-femina	11·4	9	47	5·43	9
Pteridium aquilinum	7·7	33	26	4·76	14
Gymnocarpium dryopteris	9·1	26	35	4·73	12
Blechnum spicant	8·3	29	16	4·58	18
Thelypteris phegopteris	8·9	20	53	4·52	12
Dryopteris spinulosa	7·3	140	21	4·10	19
,, *filix-mas*	5·9	12	23	3·67	50
Polypodium vulgare	3·6	21	40	2·88	80
Dryopteris spinulosa	4·3	42	26	2·48	68

A single exception is the case of *Dryopteris spinulosa*, one specimen of which contained 140 mg. of manganese. This was the only fern collected from a conifer wood and may be compared with *Plagiothecium elegans*, which had the highest level of manganese among the mosses.

Grasses (Table III). Of the grasses selected for analysis most attention has been given to three species characteristic of different soil types. *Brachypodium sylvaticum* is a species of flushed brown earths, *Holcus mollis* is most frequently found on moderately leached soils, and *Deschampsia flexuosa* is one of the dominant plants on the most acid and heavily leached podzolic soils.

TABLE III

Grasses

				Soil.	
Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	pH.	Loss on ignition % dry wt.
Melica uniflora	8·8	16	21	7·06	—
Sesleria caerulea	6·0	68	28	6·80	—
Brachypodium sylvaticum	7·2	82	18	6·99	—
" "	7·9	16	28	6·24	30
" "	8·8	0	29	6·18	21
" "	8·1	11	8	5·75	27
" "	9·8	9	23	5·49	11
" "	9·0	39	11	5·34	11
" "	6·6	16	29	5·06	14
" "	7·4	64	43	4·80	15
" "	5·1	26	20	4·73	9
Deschampsia caespitosa	9·0	34	25	5·34	27
Melica uniflora	8·5	15	22	5·24	11
Holcus mollis	8·1	89	14	4·57	18
" "	5·7	35	13	4·15	20
" "	12·0	28	18	3·92	17
" "	8·0	47	25	3·90	24
" "	8·3	44	29	3·85	46
" "	5·4	56	18	3·72	52
Festuca sylvatica	5·2	36	18	4·22	20
Deschampsia flexuosa	4·8*	10	54	5·60	42
" "	7·0	63	67	4·70	19
" "	6·9	171	57	4·22	32
" "	6·9	240	54	3·95	18
" "	5·4	48	15	3·64	9
" "	5·8	123	110	3·60	61
" "	7·1	6	7	3·38	43
" "	5·6	145	58	3·38	75

* From atypical habitat.

Deschampsia flexuosa shows the greatest uptake of both elements. The upper limit of manganese in this species is 240 mg., and of iron 110 mg. *Holcus* generally exceeds *Brachypodium* in manganese, ranging from 28 to 89 as compared with 0 to 82 mg.; while iron is roughly similar in both species and is always below 44 mg.

Although *Deschampsia flexuosa* usually contains fairly large amounts of both elements, it appears able to grow normally with relatively low levels of iron and manganese in its tissues. For example, one collection, from an abundant growth on an acid leached soil with a grey A_2 horizon, contained only 6 mg. of manganese and 7 mg. of iron per hundred grammes dry weight. This suggests that most of the available soil reserve of the two elements had been leached beyond reach of the grass roots.

Herbs (Table IV). Here again three species have been given special attention. *Mercurialis perennis* is perhaps the most characteristic plant of the flushed habitats, while *Digitalis purpurea* and *Solidago virgaurea* grow over the whole range of woodland soils. The results, however, are not very informative.

TABLE IV

Herbs

				Soil.	
Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	pH.	Loss on ignition % dry wt.
Primula vulgare	15·8	22	21	7·33	—
Geranium robertianum	7·4	46	46	6·50	—
Mercurialis perennis	17·8	23	12	7·15	—
,, ,,	13·4	0	24	6·12	19
,, ,,	15·8	8	44	5·51	19
,, ,,	12·5	9	54	5·44	16
,, ,,	12·4	7	42	5·40	20
,, ,,	14·5	41	82	5·14	14
Digitalis purpurea	12·1	1	30	6·70	—
,, ,,	15·1	97	51	4·56	27
,, ,,	12·7	64	68	4·40	20
,, ,,	8·0	41	41	4·16	26
,, ,,	6·4	41	50	4·20	33
,, ,,	7·3	88	22	4·00	21
Solidago virgaurea	10·2	14	9	6·80	—
,, ,,	11·3	120	36	4·72	13
,, ,,	10·1	49	18	4·60	52
,, ,,	10·1	46	33	4·49	22
,, ,,	8·8	48	24	4·40	29
,, ,,	10·1	53	23	4·25	34
Primula vulgaris	10·4	21	47	4·62	15
Oxalis acetosella	8·8	26	19	4·04	14
Melampyrum pratense	6·4	51	33	3·28	87

There is no strong accumulation of either element, manganese being always below 121 mg. and iron less than 82 mg. Of the various plant groups studied, herbs have the greatest mineral intake as shown by ash content. It would appear, then, that iron and manganese in this group form a relatively less important proportion of the total mineral consumption than in any other green plants.

In five out of six cases *Mercurialis* has more iron than manganese, the latter being always under 42 mg. and generally less than in the other two species. In all six samples of *Solidago*, on the other hand, manganese exceeds iron.

Woody plants (Table V). The most evident feature of this group of plants is the high level of manganese in most species, coupled with very low figures for iron. Manganese ranges up to 386 mg., and seven samples exceed 100 mg., while iron never rises above 43 mg.

TABLE V

Woody Plants

				Soil.	
Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	pH.	Loss on ignition % dry wt.
Taxus baccata	7·8	130	7	7·56	—
Fraxinus excelsior	9·5	24	27	7·16	—
,, ,,	9·7	37	12	6·25	—
,, ,,	9·1	22	44	5·41	18
,, ,,	9·7	34	17	4·90	16
Sorbus aucuparia	6·8	26	11	5·68	—
,, ,,	3·6	5	13	4·53	12
,, ,,	5·8	21	11	4·42	18
Corylus avellana	4·7	114	14	4·45	10
Quercus petraea	3·9	386	20	4·89	18
Fagus sylvatica	3·5	140	18	4·80	14
Betula pubescens	3·7	170	14	3·98	27
,, ,,	3·9	41	13	3·18	69
Myrica gale	4·2	209	12	3·82	7
Calluna vulgaris	4·1	3	31	3·82	35
Vaccinium myrtillus	5·1	246	10	3·07	63

Fraxinus, the most typical tree on the flushed soils, has a relatively low manganese content. Strangely enough, the same is true of *Sorbus aucuparia*, which usually accompanies oak on the acid soils.

It may be pointed out that these results contrast with those of McHargue and Roy (1932), who found only 3 of 23 species of trees accumulated manganese in excess of iron. All of their species, however, were growing on soils derived from limestone.

TABLE VI

Fungi

Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.
On flushed brown earths			
Amanita rubescens	12·8	<2	11
Russula cyanoxantha	11·0	2	6
Paxillus convolutus	9·4	3	12
Cantherella cibarius	8·8	2	9
Russula adusta	7·6	<1	8
,, *nigricans*	7·2	1	5
Boletus elegans	7·2	<1	5
On leached podzolic soils			
Amanita mappa	10·7	<4	12
,, *muscaria*	9·5	3	9
Lactarius quietus	9·0	<2	14
Russula nigricans	7·8	1	5
Hypholoma fasciculare	6·1	5	29

Fungi (Table VI). Samples of fungi were collected because it was thought that a group which does not photosynthesize might furnish an interesting comparison with the green plants. Unfortunately clear hyphae could not be obtained pure and in quantity, so that fruiting tops had to be used. For this reason the results may not be strictly comparable.

On this basis, while the ash content is fairly high the upper limits of intake of iron and manganese are remarkably low in comparison with green plants, being 29 mg. for the former and 5 mg. for the latter. In every case iron exceeds manganese. There is perhaps a very slight tendency towards a higher uptake on the leached soils.

II. *Plants from wet soils*

Aquatic plants (Table VII). Among the plants on wet soils this group takes up the greatest amounts of both iron and manganese, and also shows the highest total mineral uptake, as may be seen from the high ash content of the species. There is, however, a great deal of variability in both elements and no definite trend towards selective absorption of either by the group as a whole.

TABLE VII

Aquatic Plants

Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	Soil. pH.	Soil. Loss on ignition % dry wt.
Nitella sp.	16·6	105	173	6·44	2
Littorella uniflora	20·4	38	175	6·40	2
Isoetes lacustris	20·0	64	7	7·09	5
Nuphar lutea	11·4	76	26	5·58	11
Littorella uniflora	21·4	70	105	5·98	13
Potamogeton perfoliatus	17·0	48	55	6·80	21
,, *crispus*	15·5	185	82		
,, *natans*	9·4	70	33	6·54	25
,, *alpinus*	15·3	288	303	6·52	28
Sparganium minimum	20·1	378	562		
Elodea canadensis	19·8	244	132		

The highest figures are those for *Sparganium minimum* and *Potamogeton alpinus*, species characteristic of the most organic lake muds. The former contains 378 mg. of manganese and 562 mg. of iron, while the latter has 288 and 303 mg. respectively. It is on these sites, rich in reduced and soluble iron and manganese, that one might expect both elements to be most easily available to plants.

It is perhaps possible that some of the iron and manganese is precipitated on the leaves of these underwater plants, although this could not be observed. Since only normal healthy leaves were selected, such precipitation if it did occur would no doubt be of much physiological importance.

Mosses from semi-aquatic soils (Table VIII*a*). It is again very noticeable that in wet soils as in woodland, mosses assimilate much more iron than manganese. In the present case the content of iron is at least twice that of manganese in most instances. The upper limits are 307 mg. of iron and 171 mg. of manganese, both figures referring to a specimen of *Hypnum cordifolium* from the drift zone of a fen. *Sphagnum plumulosum* contains the smallest amount of iron and is also low in manganese.

TABLE VIII*a*

Mosses from Semi-aquatic Soils

Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	Soil.	
				pH.	Loss on ignition % dry wt.
Sphagnum plumulosum	3·6	9	34	6·63	45
,, ,,	6·3	4	26	6·53	88
,, *auriculatum* v. *ovatum*	8·8	4	260	6·09	3
,, *teres*	5·6	21	88	5·91	60
,, *fimbriatum* v. *laxifolium*	4·1	8	66	4·00	93
Hypnum cuspidatum	5·0	28	160	7·32	79
,, *cordifolium*	3·5	131	307	5·76	65
Thuidium tamariscinum	5·0	20	130	4·85	80

TABLE VIII*b*

Other Plants on Semi-aquatic Soils

Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	Soil.	
				pH.	Loss on ignition % dry wt.
Typha angustifolia	3·9	60	19	6·30	22
,, *latifolia*	9·3	87	32	5·49	17
Sparganium erectum	11·9	87	24	5·90	26
Juncus effusus	2·8	31	7	5·28	31
Narthecium ossifragum	6·6	99	26	4·20	96
Eleocharis palustris	6·3	100	44	6·00	7
Cladium mariscus	2·9	11	12	6·58	75
Carex lasiocarpa	5·5	46	18	5·38	66
,, *vesicaria*	6·0	55	19	4·46	56
,, *canescens*	5·5	148	65	4·40	80
,, *paniculata*	5·3	82	26	4·37	57
,, *inflata*	5·0	55	18	3·91	86
Molinia caerulea	4·7	9	11	5·70	25
,, ,,	4·5	27	3	5·84	53
,, ,,	4·2	12	12	5·06	81
Glyceria fluitans	7·6	38	31	5·85	17
Calamagrostis lanceolata	5·6	32	16	5·28	37
Phalaris arundinacea	6·3	63	34	4·68	21
Phragmites communis	6·4	23	23	5·08	70
Menyanthes trifoliata	10·1	28	16	5·86	86
Filipendula ulmaria	8·3	120	23	5·48	55
Salix aurita	6·5	272	17	4·81	18
,, *fragilis* v. *decipiens*	6·6	130	37	4·77	26
,, *atrocinerea*	4·7	102	26	5·35	64
Alnus glutinosa	5·1	80	24	4·63	42

Other plants from semi-aquatic soils (Table VIII*b*). On these soils, which range from neutrality to pH 4 and from silt to peat, manganese uptake by plants other than mosses reaches far greater heights than does that of iron. The top values are 272 mg. of the former and 65 mg. of the latter. There is little correlation of accumulation with pH or organic content of the soil.

Trees are rather high in manganese, having from 80 to 272 mg. Grasses appear to be relatively low, with from 9 to 63 mg.

Raised-bog plants (Table IX). On these sites the species sampled (which unfortunately do not include any mosses) all absorb more manganese than iron. Figures run from 35 to 50 mg. of the former and from 10 to 21 mg. of the latter. Accumulation by the plants on these oxidized peats is rather low in comparison with plants on the other wet soils, as is the total mineral intake measured by ash content.

TABLE IX

Plants from Oxidized Raised-bog Peats

Species.	Ash % dry wt.	Mn mg. per 100 g. dry wt.	Fe mg. per 100 g. dry wt.	Soil. pH.	Soil. Loss on ignition % dry wt.
Eriophorum vaginatum	2·4	47	10	3·18	98
,, *angustifolium*	2·4	57	16	3·18	97
Scirpus caespitosus	2·7	43	21	3·70	92
Molinia caerulea	3·3	59	12	2·98	94
Deschampsia flexuosa	4·4	35	18	2·86	95
Myrica gale	2·9	53	12	3·91	86

DISCUSSION

One of the notable features of this investigation is the high proportion of both iron and manganese present in natural vegetation. The values for manganese are much greater than those recorded by Goodall and Gregory (1947) for crop plants. The reasons for this are possibly to be found in the differences in natural and cultivated soils. The former are generally more acid, and compounds of iron and manganese are usually more soluble at higher hydrogen-ion concentrations. Natural soils also tend to contain more humus and therefore have a greater adsorptive capacity. Both elements may be adsorbed on the humus colloids and thus prevented from precipitating as ferric hydroxide or manganese dioxide. In addition the probable formation of stable and soluble organic complexes should not be overlooked.

No close correlation of accumulation of either element with soil pH could be observed in those plants of which several samples were collected. This is contrary to the findings of Olsen (1934), who obtained a good correlation of manganese in individual woodland species with the pH of the soil in which they were growing. It seems likely that the high degree of relief and marked variability in microtopography of the Lake District woodlands might lead to large differences in local leaching intensity and therefore in the concentration of the elements in the soil. In this connexion we may note that the Danish sites do not show the extremes of acidity found in the Lake District. In the latter locality, then, the absolute amount of available minerals may vary more and therefore play a larger part in determining plant absorption. That manganese assimilation is much greater at higher concentrations has been clearly shown by Olsen in the laboratory. It is probably generally true of iron also. For example, a green alga (*Chlorella vulgaris*) shows a direct correlation of iron content with supply of this element in the culture medium, as may be seen from Table X.

TABLE X

Relation of Iron Uptake by Chlorella vulgaris *to Iron Supply in the Culture Solution*

Fe supplied p.p.m.	Ash % dry wt.	Uptake, Fe mg. per 100 g. dry wt.
0·05 to 0·10	4·7	5
	5·8	4
	6·3	6
0·10 to 0·50	4·2	21
	5·6	22
	6·1	21
1·0	5·0	45
	6·6	45

It is interesting to observe that marked accumulation of manganese by the Danish plants begins below about pH 5. This value was determined colorimetrically and corresponds to a lower figure, possibly about pH 4·7, using a glass electrode and the sampling methods employed by us. The latter figure lies on the borderline between flushed soils with *mull* humus and leached soils with *mor* humus in the Lake District.

It should be of great interest to determine whether there are any differences in the uptake of iron or manganese by plants in general on the brown earths and the leached podzolic soils. In order to compare series of such variable results a derivative of the frequency curve has been employed. The method consists in plotting, for a number of levels of the element considered, the percentage of the total number of samples whose content lies on and below each given level. In this way we may obtain a summation curve, generally sigmoid in form, which gradually approaches 100 per cent. as the maximum level of the element is increased. For example, 60 per cent. of the green plants sampled contain less than 30 mg. of iron per hundred grammes dry weight, while 76 per cent. have less than 50 and 96 per cent. less than 180 mg. If one curve lies below another at a given level of the element it is evidence of a higher percentage of samples with a content above that level. Again in illustration, 60 per cent. of all green plants sampled have less than 30 mg. of iron, while 41 per cent. have less than 30 mg. of manganese. Conversely, only

40 per cent. of the green plants have more than 30 mg. of iron, while 59 per cent. have more than 30 mg. of manganese. An examination of Fig. 2, plotted in this way, shows that manganese in plants from leached soils exceeds that in plants from flushed soils.

In the present instance all green plants which were growing on woodland soils of pH less than 4·5 were taken as representative of uptake on the leached soils; those on soils above pH 5·0 were considered to be typical of accumulation on the flushed soils. The data are weighted unequally in that where several samples of a species were collected all are included, as an average value of such variable results would be meaningless. Nor are the numbers of species from the different plant groups equal on the two soil types. Finally, an accurate assessment of the specific composition of plant communities on the two habitats is lacking. For these reasons the results should be taken as merely suggestive of certain relationships, and not in any way conclusive.

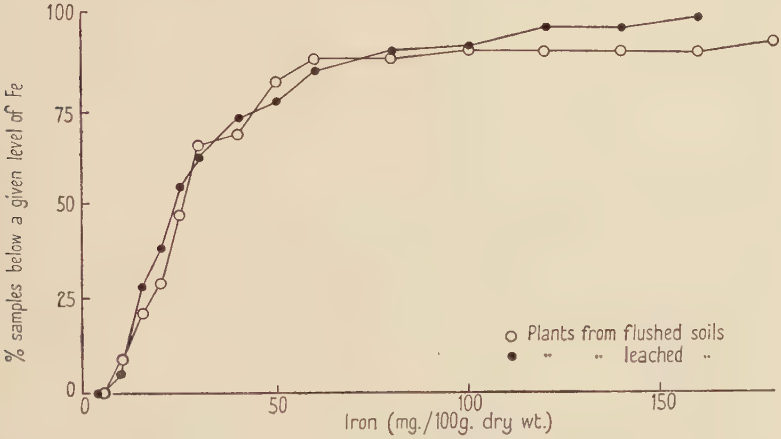

FIG. 1. Iron in green plants from flushed and leached soils.

In Fig. 1 is shown the uptake of iron by green plants on the two woodland soil types. In Fig. 2 the results for manganese are presented. With the above limitations in mind, the curves suggest that for the plants considered iron uptake is much the same on the two soils, whereas the accumulation of manganese is far greater on the leached than on the flushed habitats.

By inspection of the figures it may also be seen that on the leached soils the content of manganese in the plants examined greatly exceeds that of iron. On the flushed series the reverse is true, though there is little significance in the difference between the two elements.

These results are somewhat similar to those of Olsen's (1934) for beech leaves. He found little difference in the iron content of leaves from trees on acid and neutral sites, while manganese varied greatly. On the neutral soils the quantity of manganese in the leaves was usually slightly less than that of iron; whereas on acid soils manganese was much in excess of iron. Erkama

(1947) found plants from acid woodland to contain more manganese than iron, while garden plants on alkaline soils assimilated iron in excess of manganese.

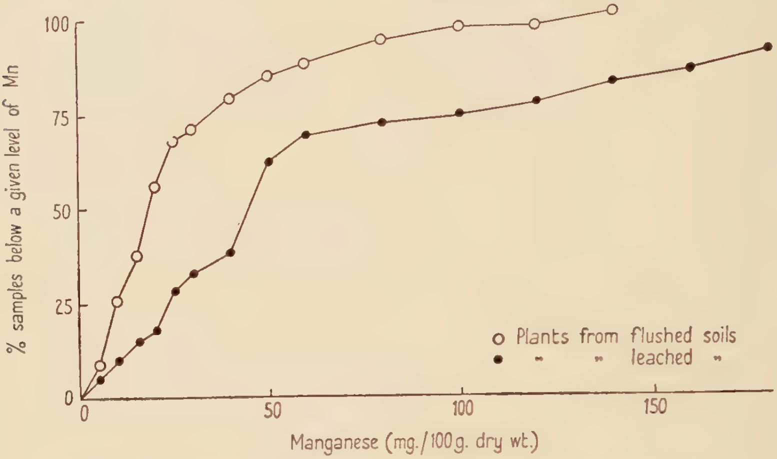

FIG. 2. Manganese in green plants. From flushed and leached soils.

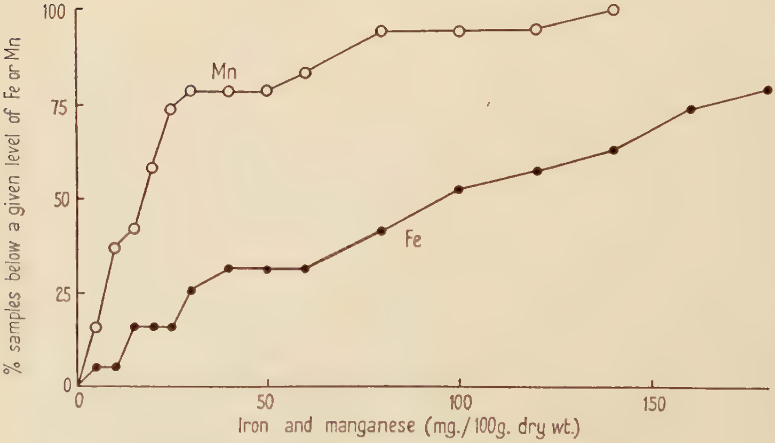

FIG. 3. Uptake of manganese and iron by mosses.

Where supplies are readily available the amounts of iron or manganese taken up depend on the plant. For example, mosses accumulate iron in large quantities, while trees on the same soils appear to absorb manganese in preference to iron. This is very clearly shown in Figs. 3 and 4. It is interesting that on the soils investigated here fungi and mosses, the simplest plants studied, appear to absorb iron selectively, while the general trend among the 'higher' plants is in the reverse direction, though perhaps not so strongly marked. In this connexion it may be noted that a single lycopod species from

Scottish limestone, *Selaginella selaginoides*, also showed a great accumulation of iron, 550 mg. as against 33 mg. of manganese. The only plants found to take up both elements in large amounts were certain of those on the strongly reducing lake muds.

On the other hand, most species can probably grow normally with far less than their usual intake of these micro-nutrients. As an illustration we may again consider *Deschampsia flexuosa*. This species can accumulate well over 100 mg. of manganese. However, an abundance of healthy plants on a heavily leached knoll contained only 6 mg. of this element. A similar picture is given with regard to iron by the moss *Dicranum majus*. It is not clear whether the characteristic high contents of iron or manganese in some species are due to a selective metabolic process or to accumulation because mechanisms to exclude them are lacking. They appear at any rate to be something of a luxury consumption. The small amounts of both elements in the fungi may suggest that there is a possible connexion between photosynthesis and the absorption of iron and manganese in large quantities.

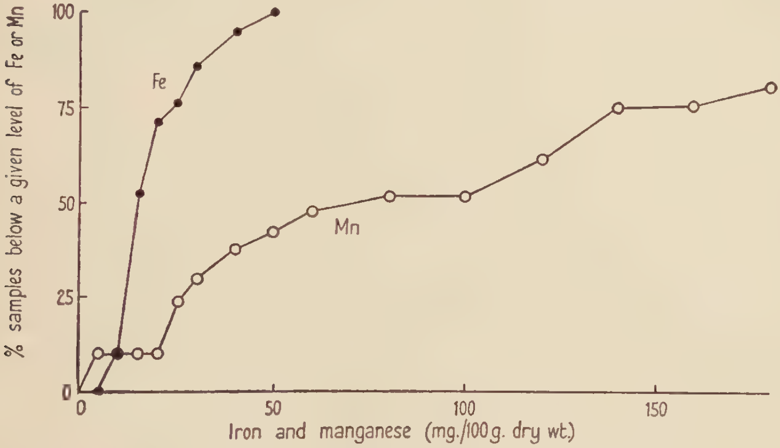

FIG. 4. Uptake of manganese and iron by woody plants.

Certain workers have stated that the ratio of iron to manganese may be of great importance (Somers and Shive, 1942). However, this claim is based on the determination of *active* iron and manganese, which is an empirical concept whose value is not clearly established. The fact that plants on acid soils accumulate manganese rather than iron, while on circumneutral soils the reverse is more likely, might be taken to support the idea of ratio importance, but may equally possibly reflect soil availability. In contrast the fact that mosses selectively absorb iron and trees manganese probably indicates a real metabolic peculiarity. In spite of these considerations, a perusal of the data for species of which several samples have been taken shows a wide variation both in the amounts absorbed and in the ratio, without apparent effect on the health or abundance of the plants. It is probably true to say that while certain species tend to absorb more of one element than the other, both the absolute amounts and the ratio of one to the other may apparently vary a good deal without harm to most plants.

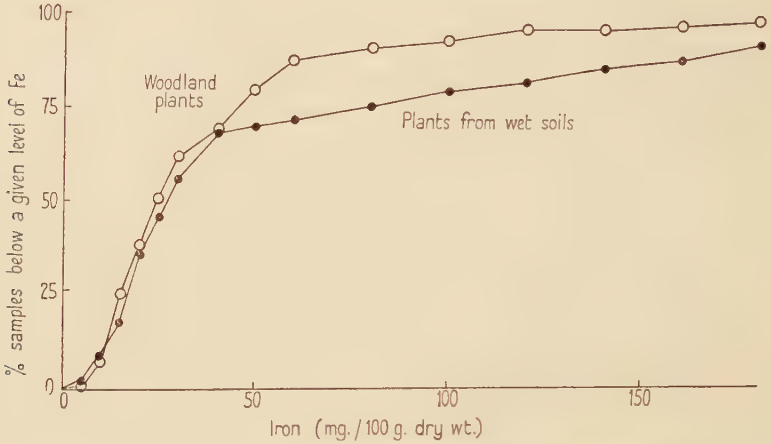

FIG. 5. **Iron in green plants from woodland and waterlogged soils.**

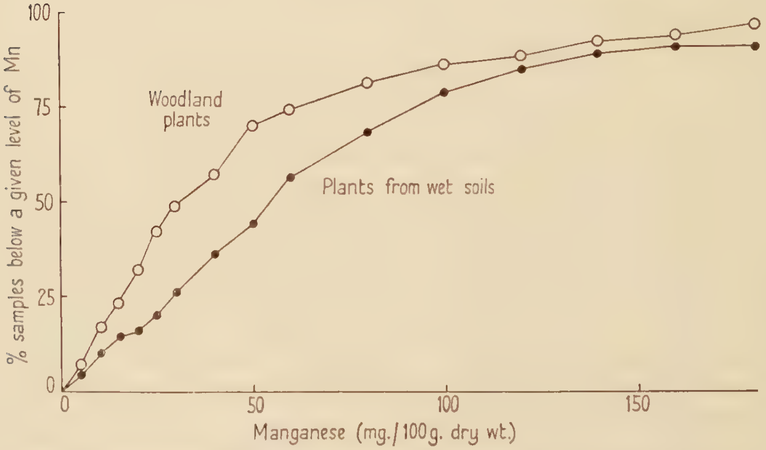

FIG. 6. Manganese in green plants from woodland and waterlogged soils.

As a further point of interest, we may compare by the graphical method the plants of wet soils with those from woodland. This has been done in Figs. 5 and 6. It might be expected that the plants from the wet soils which are often reducing would take up more iron and manganese than those on the woodland soils with a high redox potential. This is in fact true of manganese. With regard to iron, the curves are similar except at the higher levels, where the very high figures for the aquatic plants bring the line for wet soil plants well below that for woodland. On the wet soils as in woodland, manganese accumulation generally exceeds that of iron on wet soils.

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Reproduction in *Halopteris* (Sphacelariales)

BY

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With fifty-four Figures in the Text

ABSTRACT

Oogamy is recorded in five southern species of *Halopteris*. In each the gametangia and asexual sporangia occur in similar sori, although situated on separate plants. The plurilocular antheridia and the unilocular oogonia occur within one and the same sorus. In both the dehiscence is apical.

In *H. congesta* fertilization has been observed in cultures. The polarity of the zygote is established by the direction of the first-formed wall. Early divisions convert it into a nodule composed of small cells. First rhizoids appear and then cylindrical shoots arise from the opposite pole. Sporelings of five New Zealand species of *Halopteris* have been raised in culture. The conditions in the culture greatly affected the character of the sporelings, the simplest of which at the end of 2 months' growth were only uniseriate filaments. The best developed sporelings had a compact basal disc with up to four upright shoots showing the beginnings of branching.

A PRELIMINARY note (Moore, 1946), accompanied by a photograph, has already recorded experimental confirmation of Sauvageau's belief that oogamy occurs in *Halopteris*. The present paper offers more details about the life-history of certain species of this genus of Sphacelariales. Oogamy undoubtedly occurs in three species of *Halopteris* found in New Zealand, and in three or four found in Australia. Specimens of *Phloeocaulon spectabilis* from Robe, South Australia, show 'plurilocular sporangia' resembling the antheridia of *Halopteris*. Vegetatively and in the position and arrangement of the sori of reproductive organs, asexual and sexual plants of one and the same species resemble one another very closely, but gametangia and sporangia are always borne on separate plants.

MATERIAL AND METHODS

Fresh material for cultures was obtained mostly from within a few miles of Wellington city, e.g. at Lyall Bay, Island Bay, and Ohariu Bay; one lot came from Campbell's Bay, Auckland. For cultures filtered sea-water in clean glass vessels was used. The sea-water was collected from rocks exposed at low tide on projecting points on the north shore of Cook Strait where there is a rapid passage of ocean water with an average temperature, between July and October, of 10–12° C. In many respects this will have the advantages of

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that known as 'outside' water at Plymouth; at the same time it always came from places near which other brown seaweeds were growing. Kylin (1941) and Suneson (1942) have shown that surface water from the *Fucus-Ascophyllum* zone can give better results than either surface or deep water from farther off shore.

Petri dishes or squat cylindrical jars, 8 cm. in diameter and 5 cm. high, gave better results than the 100-ml. beakers that were also tried. Growth in the cultures would probably have continued much farther if larger vessels had been used and the water changed more frequently. The culture vessels were lightly covered with glass plates or placed uncovered on a glass sheet under an inverted pneumatic trough. Direct sunlight was avoided, but otherwise the cultures were exposed to the full light of a northerly window. For catching the heavy ova, slides placed in the vessels must be almost horizontal.

As a matter of convenience the main culture work was carried out in the winter months. Asexual plants are found throughout the year. Sexual plants have been collected in every month except June, but they are much more plentiful from July to the end of October. Drawings were made on graph paper, with the help of a square-lined eyepiece; those of embryos were mostly prepared from living plants.

Besides material grown in cultures, fresh plants were examined frequently during 1945–6 and in 1947, and less frequently after that. Both from Wellington and from other parts of the coast bulk collections, together weighing many pounds and containing many hundreds of plants, have been carefully searched through to provide a reliable basis for records of presence and absence. A careful study has been made of all dried *Halopteris* specimens in the herbaria of the Auckland and Dominion museums, in the collections of R. M. Laing and W. A. Scarfe, as well as in the Botany Division Herbarium. Mr. V. W. Lindauer contributed some particularly interesting specimens. The New Zealand material of *Halopteris* in the Melbourne National Herbarium has been seen on loan, while Mr. H. B. S. Womersley has lent a considerable number of sheets from South Australia.

SEXUAL REPRODUCTION

Sexual plants of *H. congesta* (Rke.) Sauv., *H. hordacea* (Harv.) Sauv., and *H. brachycarpa* Sauv. (?) are known in New Zealand. Apart from characteristic differences in arrangement, the gametangia show great uniformity in details of structure and development. This has also been observed in *H. hordacea*, *H. spicigera* (Aresch.) Moore, and *H. pseudospicata* Sauv. (?) from Australia.[1] Sauvageau records antheridia and oogonia also in *H. filicina* (Gratel.) Kütz. and in *H. scoparia* (L.) Sauv.

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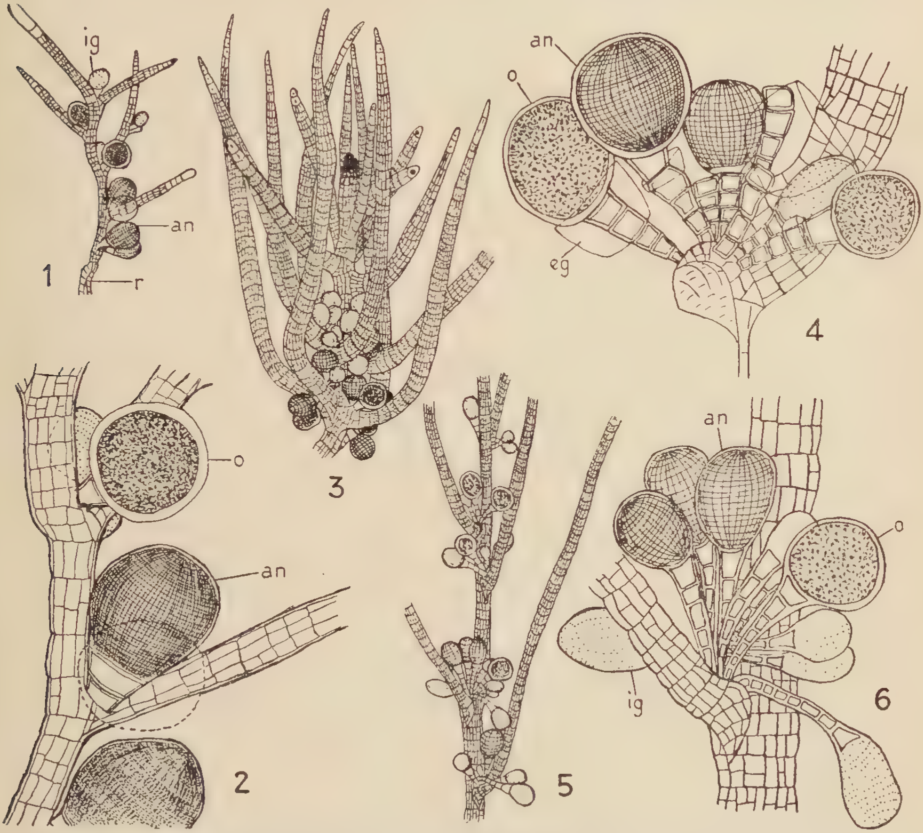
[1] Sauvageau (1904) described sexual *H. brachycarpa* and asexual *H. pseudospicata* from meagre Australian material. Without reference to the type specimens and a wider knowledge of the range of forms in Australia these determinations must remain doubtful. Sauvageau gives *Sphacelaria spicigera* Areschoug as a synonym of *H. hordacea*; the two plants seem, however, to be sufficiently distinct (Moore, 1950).

Arrangement of gametangia

The gametangia (Figs. 1–6) are always in axillary groups on pedicels that are either long, as in *H. brachycarpa* (Fig. 6), or short, as in *H. congesta* (Fig. 2), sparingly branched as in *H. brachycarpa*, or unbranched as in *H. hordacea* (Fig. 4). The subtending member may be either of strictly limited growth (e.g. *H. hordacea*, Fig. 3) or a longer unit that branches farther (e.g. *H. brachycarpa* (Fig. 5). All plants examined were monoecious, with antheridia and oogonia often mingled in the same axil; on the short arrested reproductive shoots of *H. congesta* (Fig. 1) there is a tendency for antheridia to predominate in the proximal and oogonia in the distal axils.



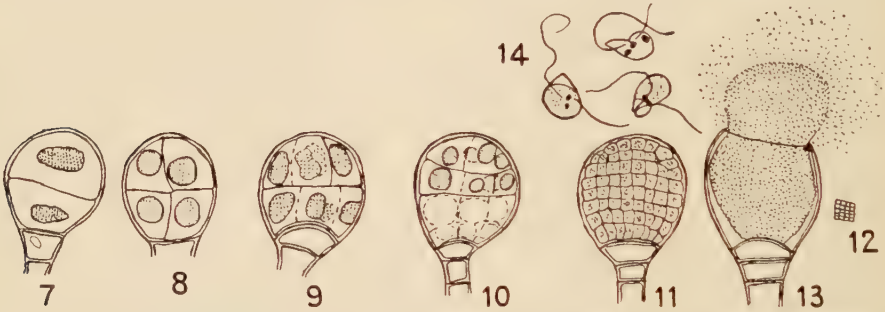
FIGS. 1–6. Gametangia of *Halopteris*. 1, 2, *H. congesta* (Rke.) Sauv.; 3–4, *H. hordacea* (Harv.) Sauv.; 5, 6, *H. brachycarpa* Sauv. (?) (1, 3, 5 × 30; 2, 4, 6 × 126). *an*, antheridium; *eg*, empty wall of gametangium; *ig*, immature gametangium; *o*, oogonium; *r*, rhizoid at base of branchlet.

Marked protogyny has been encountered only once. A seemingly purely female branch was shedding ova on July 16, but by August 5 it showed ripe oogonia and antheridia, fertilization being observed on the next day. The specimens described by Sauvageau (1904, pp. 412–13) were probably at the stage when the antheridia were not yet distinguishable.

Development of gametangia

Gametangia develop from axillary cushions. After a pedicel is initiated, the pale terminal cell swells until it reaches a diameter of about 100 μ. At this stage male and female are indistinguishable, but the antheridium becomes recognizable when it divides equatorially into two (Fig. 7). Stages in its further development are summarized in Figs. 8–11; the compartments finally measure about 4 μ (Fig. 12). The septa are thin and rarely clearly observable after the first few divisions. The mature antheridium is bright orange in colour.

The oogonium remains undivided and, when mature, its contents show superficial brown flecks. In dying plants oogonia are recognized by their dark contents, which are contracted strongly away from the hyaline wall. Even in herbarium material the antheridia may be yellow and the oogonia brown. Sometimes (Fig. 4) the pedicel grows through the dehisced oogonium (*eg*) and forms another one (*o*) farther up.



FIGS. 7–14. Antheridia and sperms of *H. congesta*. 7–11, stages in development of antheridium (× 126), preparations plasmolysed in an attempt to show walls; 12, final size of compartments (× 160); 13, dehiscing antheridium (× 126); 14, sperms (× 1180).

Dehiscence and gametes

Dehiscence of the gametangia has been observed only in sexual plants from the Wellington district, most of which, if not all, probably belong to *H. congesta*. In a fairly strong light the antheridium bursts apically and the contents emerge as an orange mass (Fig. 13), from the surface of which sperms soon swim actively away. Each oogonium liberates a single ovum that becomes slightly constricted as it emerges through the irregular apical aperture. The heavy, non-motile ova sink immediately to the bottom of the dish.

The sperms (Fig. 14) have an average diameter of about 4 μ and are colourless except for two, three, or possibly four minute chromatophores. An eyespot was not certainly seen. The narrow end is unstained by safranin, following treatment with osmic acid and tannic acid.

The globular ovum is pale and about 100 μ in diameter. The cytoplasm is much vacuolated, and there is a single rather large nucleus.

Fertilization and early division of zygote

Fertilization has been observed several times, always before about 10 a.m. Each egg becomes surrounded by a rather well-defined zone of very active sperms (Figs. 15, 16); the majority soon disperse, but some may be recognized nearby for an hour. In most of my cultures conjugation took place between gametes from the same plant, and this must happen frequently in nature.

By the end of 12 hours after fertilization the zygote, now enclosed within a wall, has divided into two equal cells, while after 24 hours the young embryo consists of four and sometimes of as many as eight cells. The first rhizoids become recognizable during the 3rd day and by the end of the 4th day the first cylindrical shoot has appeared. The central tubercule (Sauvageau, 1904, p. 430) or nodule (cf. Fritsch, 1945, p. 317), now multicellular and densely pigmented, remains about the same size as the zygote. Figures 15–19 show successive stages in the development of several embryos, while Fig. 20 shows the sequence of divisions diagrammatically.

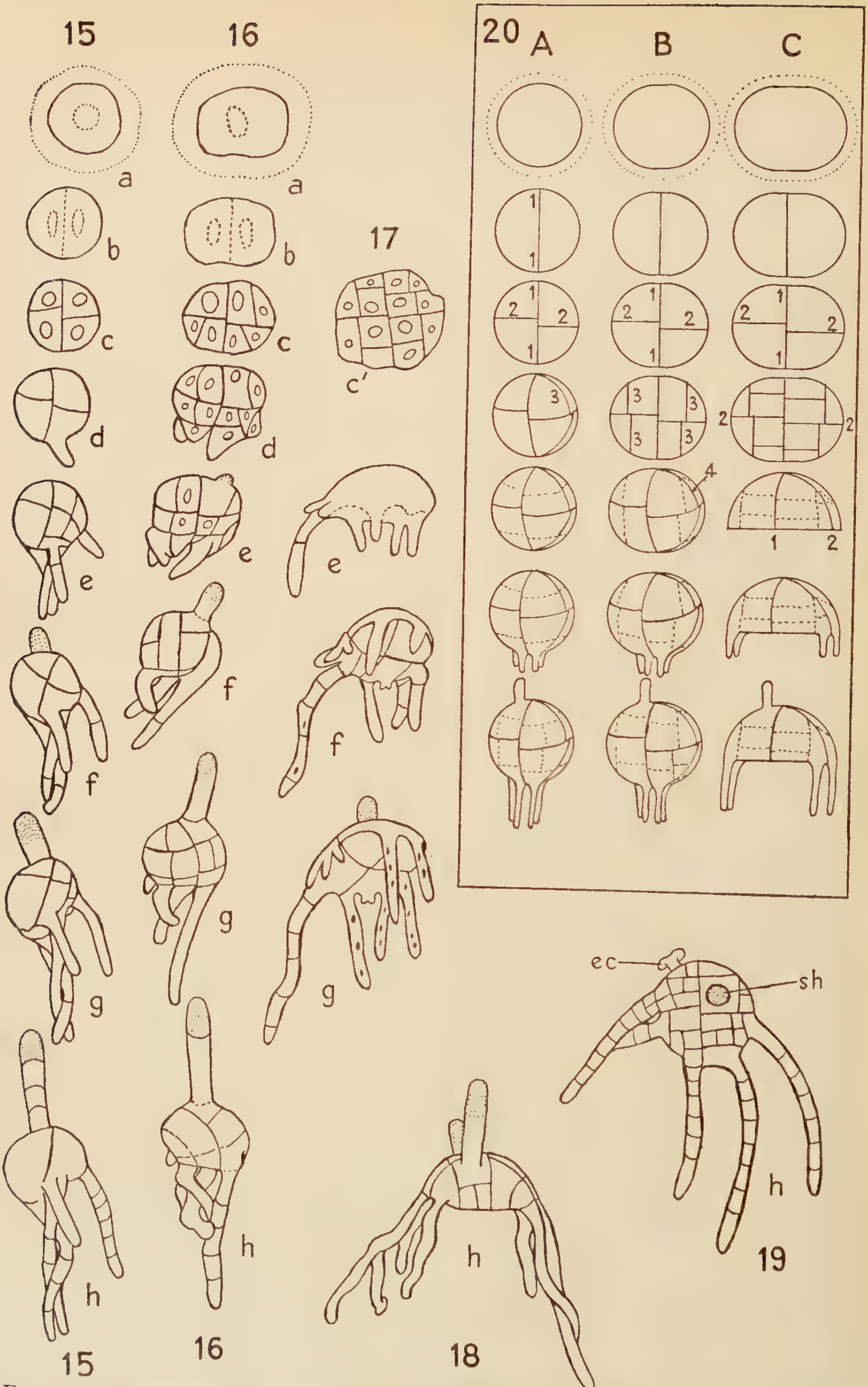
In certain instances the formation of an envelope around the ovum was followed by only one or two divisions before growth was arrested; if the culture as a whole was doing well, it was suspected that such ova were unfertilized.

Germling development (Figs. 15–23)

Usually, and probably invariably in healthy embryos, the first wall (Fig. 20, 1–1) defines the mid-vertical plane. Where the egg is globular at the time of fertilization (Type A, Figs. 15, 20 A), the next walls (2–2) are horizontal and the third set (3–3) vertical and at right angles to the first two. After further divisions, rhizoids grow out from the cells at one pole, and soon after a shoot develops from one of the four cells at the opposite pole.

Frequently, in cultures at least, the egg at the time of fertilization is already somewhat flattened, and the sequence of division then depends on the degree of flattening. If this is slight (Type B, Figs. 16, 20 B), the second walls (2–2) again define the embryo's horizontal axis; at the time of formation, however, these second walls are not, as in Type A, parallel to the horizontal substratum, but are perpendicular to it and to the plane of flattening. Further divisions in planes parallel to 1–1 or 2–2 or to both may follow; only when the embryo becomes thicker are walls formed at right angles to these earlier ones and parallel to the substratum. Embryos from flattened zygotes of Type B thus develop lying on their sides, and rhizoids and shoot, developing as in Type A, lie at first parallel to the substratum. The growing rhizoids generally lift the embryo from its horizontal position until the shoot becomes approximately erect.

When the egg is still more flattened the first and second walls are both vertical in relation to the resulting embryo, and several divisions parallel to them often follow before the first horizontal divisions parallel to the substratum occur (Type C, Figs. 17, 20 C). Rhizoids then develop around the periphery of the hemispherical embryo, usually first from the cells bordering 2–2. The embryo, suggestive of a tortoise when viewed from above (Fig. 19), is gradually



FIGS. 15–20. Formation of early embryos in *H. congesta.* 15, 16, development of two adjacent plants on one slide; 17, 18, two plants from another part of same slide; 19, plant from another culture from the same twig (×90). *a,* egg with halo of sperms; *b,* after 12 hours; *c,* after 24 hours; *c′,* after 32 hours; *d,* 3rd day; *e,* 4th day; *f,* 5th day; *g,* 6th day; *h,* 7th day; *ec,* swollen end cell of rhizoid; *sh,* shoot; 20, diagrammatic representation of development of germling: A, like Fig. 15; B, like Fig. 16; C, like Figs. 17, 18, and 19.

elevated as the rhizoids lengthen, and not uncommonly falls over on to one side (Figs. 17, 18). The first shoot develops from one of the four 'top' cells, even if these happen to be pointing downwards at the time (Fig. 17 *g*).

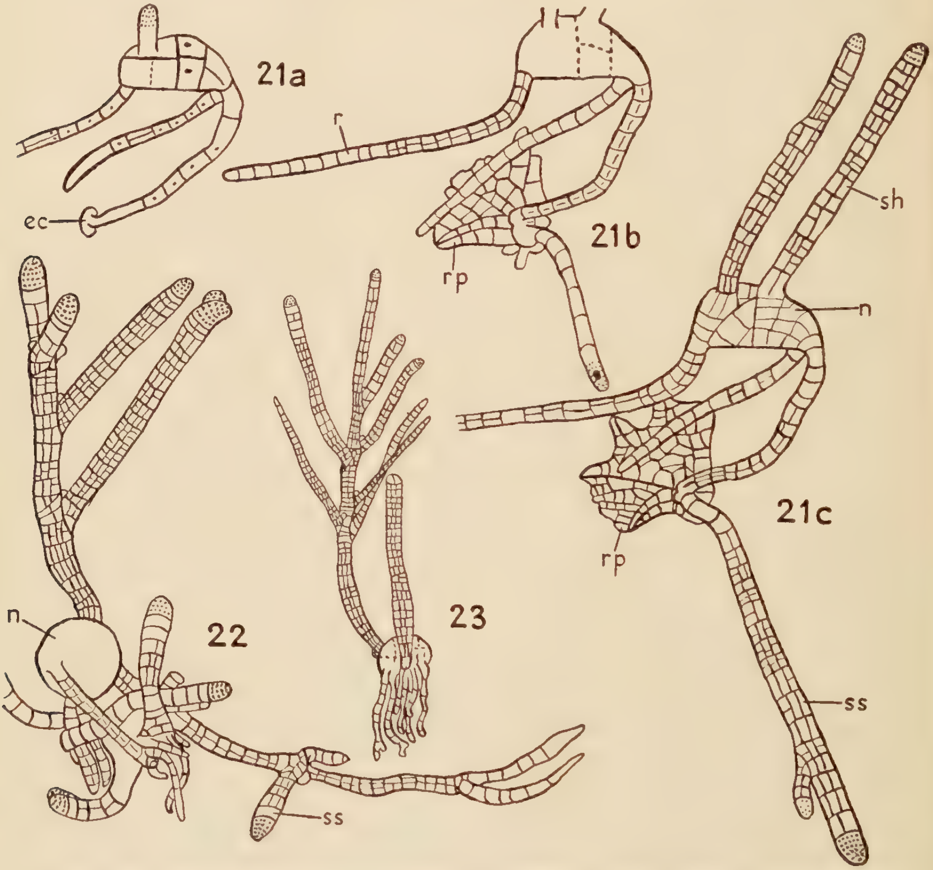
Whether the embryo is free or erect as in Type A, lies on its side as in Type B, or has a flat base as in Type C, the first wall always occupies a plane traversing the two poles, instead of dividing the zygote into an upper and a lower cell as in fucoids. The rhizoids may be displaced outwards as in Type C, but the first shoot seems always to develop from one of the quadrant of cells surrounding the opposite pole (Fig. 19, *sh*). Two of the quadrants may produce shoots, but more than two have not been noted. The number of rhizoids is far more indefinite.

No experiments were made to find out what determined the direction of the first septum which establishes the polarity of the embryos; it was, however, noted that, in undisturbed cultures with unilateral lighting, shoots tended to grow towards the light, rhizoids away from it. In Type C embryos the shoot always arises from the convex side, even if the orientation of the germling is changed after only two or three walls have been laid down. Types A and B often showed no uniform direction of growth; for example, three embryos of the same age attached to a single slide within an area of little more than 2 sq. mm. had shoots pointing towards 3, 8, and 11 o'clock respectively. In the few plants where an embryo was seen developing within an oogonium, the shoot projected from the top of the wall.

Older stages of germlings are seen in Figs. 21–3. The young rhizoids soon become septate and resemble the adult ones, the longest seen in cultures being 5 mm. long. Shoots soon show the large apical cell typical of the family, while their older cells divide longitudinally and transversely. Early branching is in several planes, the most highly branched plant seen in the cultures being that shown in Fig. 22. Such plants continued to grow for 2 months if the sea-water was changed occasionally, and survived for 1 or 2 further months without developing any more.

As pointed out by Sauvageau (1904, p. 430, Fig. 88), germlings are frequently found entangled in the sexual spikes of *H. hordacea* and Fig. 23 represents such a germling. It shows a later stage of branching than Fig. 22. Germlings of this kind lived quite satisfactorily in culture and there is no doubt that adult plants could originate from them. Germlings entangled in sexual plants of *H. congesta* (cf. Sauvageau 1904, p. 414) and *H. brachycarpa* are also very similar to those grown from zygotes of the former species.

Rhizoids often branch where they meet the substratum; alternatively the end cell (Figs. 19, 21 *a*, *ec*) may enlarge and then divide, giving rise to a light-coloured horizontal plate (Fig. 21 *b*, *c*, *rp*), from which secondary or supplementary erect shoots (*ss*) almost always arise. This plate can be compared, both as to origin and as to method of growth, with the disc of young plants of *H. filicina* described and figured by Sauvageau (1904, p. 321, Fig. 61); there is a striking likeness, too, to the disc formed in the germination of the propagula of *Sphacelaria cirrosa* (Sauvageau, 1903, Fig. 45). A shoot may also arise directly from a rhizoid without formation of any basal plate (Fig. 22, *ss*). Anything that prevents or arrests growth of the primary shoots from the nodule induces this rhizoidal plate formation; it is regularly seen, for example, in germlings where the upper hemisphere of the zygote has suffered injury.

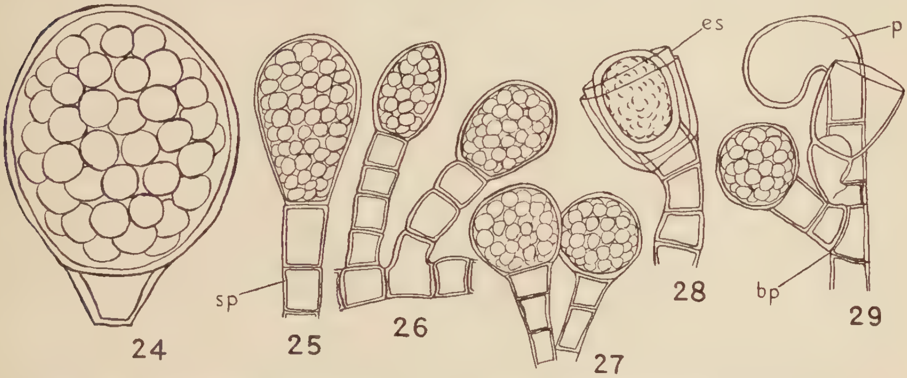


FIGS. 21–3. Development of germlings. 21, *H. congesta* (× 80): *a*, plant 7 days old; *b*, same 15 days old; *c*, same 26 days old; Type C. 22. *H. congesta* (× 80): germling about 6 weeks old. 23. *H. hordacea*, older germling found entangled in sexual spike (× 32): *n*, nodule; *r*, rhizoid; *rp*, rhizoidal plate; *sh*, shoot; *ss*, secondary shoot.

Examination of the cultures suggests that this is simply a supplementary device and that a healthy young plant can quite well develop directly from the nodule which then alone represents the prostrate part of the plant.

ASEXUAL REPRODUCTION

All New Zealand species of *Halopteris* produce unilocular sporangia fairly freely. Asexual plants of *H. spicigera*, *H. congesta*, *H. funicularis*, and *H. brachycarpa* occur abundantly about Wellington. Fresh asexual plants of *H. hordacea* have been less easily available, though they are common enough in Auckland. *H. novae-zelandiae* and a species nearly related to the Australian *H. platycena*, both with solitary sporangia, are comparatively rare in the Wellington area. The five species first mentioned have the sporangia aggregated in groups of a dozen or many more. The sori are always axillary and correspond in position with the gametangial sori of the same species. In *H. funicularis* and *H. brachycarpa* they are mostly on long branches, in *H. congesta* mostly on short reproductive shoots, and in *H. spicigera* and *H. hordacea* in terminal barley-like spikes. Pedicels are long or short, branched or unbranched, paraphyses may be present or absent, but the sporangia themselves (Figs. 24–9) are essentially alike, though differing in shape and size; typical dimensions are: *H. spicigera* 70 × 40 μ; *H. congesta* 35 × 35 μ; *H. brachycarpa* 45 × 32 μ; *H.* aff. *platycena*, markedly larger, 87 μ long.



FIGS. 24–9. Sporangia of *Halopteris* (× 310). Contents shown diagrammatically. 24, *H.* aff. *platycena*; 25, *H. hordacea*; 26, *H. brachycarpa* (?); 27, *H. congesta*; 28, 29, *H. funicularis*. *bp*, branching pedicel; *es*, empty sporangium wall; *p*, paraphysis; *sp*, simple pedicel.

Development of sporangium

Sporangia develop from axillary cushions which, as in the case of the gametangia, are subtended either by special bracts or by ordinary laterals. The terminal cell of the pedicel swells to form the sporangium. No internal walls are laid down, but the contents divide into uninucleate portions which emerge as a whole through an apical opening and then separate as zoospores. The sporangial wall persists after dehiscence, and further growth of the pedicel often produces successive crops of sporangia (Fig. 28).

The dehiscence of the sporangia takes place in the way described by Clint (1927) and Papenfuss (1934) for *Sphacelaria bipinnata.* The spore-mass emerging through the apical pore is ejected clear of the sporangium, and more than half a minute elapses before the first zoospores detach themselves and swim away. Light seems to favour dehiscence. Adjacent sporangia do not necessarily discharge simultaneously; successive dehiscences from a few sori on one small twig maintained a supply of active zoospores for more than an hour and a half.

The zoospores measure about 13 × 8 μ and have the form shown in Fig. 30. They swim actively in a hanging drop, mostly in a downward direction. Many settle within a few minutes, but a few may remain active for as much as half an hour. No fusion of the zoospores has been seen, but sometimes the mass of zoospores fails to separate completely.

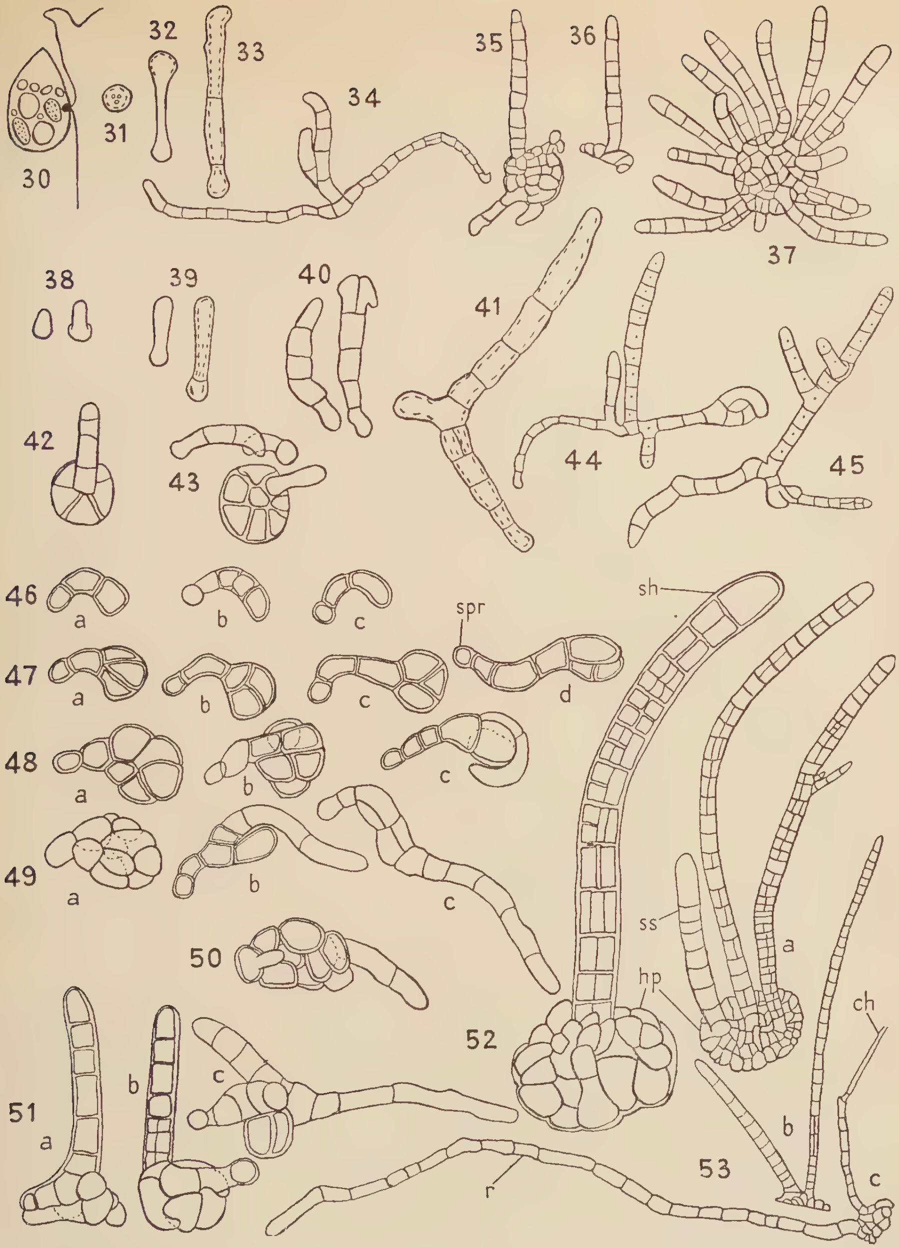
Sporelings (Figs. 31–53)

The zoospore after rounding off soon secretes a wall and rests for a few days before germinating. Fairly good sporelings have been grown from zoospores of *H. congesta*, *H. funicularis*, *H. brachycarpa*, and *H.* aff. *platycena*, but the largest ones of *H. spicigera* obtained were only a few cells long.

If the parent material is not sufficiently fresh, not all the zoospores of a sporangium become active. Several (e.g. 2–20) may cohere and settle as a flat mass where they were extruded. Each forms its own wall, attaching itself to the substratum, and all grow sufficiently to become angular as a result of mutual pressure. Often only one member of such a group germinates, but sometimes several begin to develop, simultaneously or successively. The entire group may resemble an older sporeling in a more thriving culture. It is possible that a composite plant sometimes results from the intertwined products of spores that germinate close together (Fig. 43). Fig. 37 shows a rather unusual case where a large number of spores, possibly the whole contents of one sporangium, have remained in a globular mass, on the surface of which about twenty have germinated.

Normal germination begins with a protuberance from one side of the resting spore (Fig. 38); when this has lengthened until it is at least twice as long as wide, a cross-wall arises at approximately the level of the original spore. The further growth of the filament varies considerably according to culture conditions. It may elongate to form a uniseriate filament, 30 or more cells long when 2 months old. On the other hand, the end cell may divide longitudinally or obliquely to form the beginnings of a horizontal plate (Fig. 47), as early as the three-celled stage. The long filaments may branch (Fig. 34), or even produce branched upright threads (Figs. 44, 45), but the sturdiest and healthiest sporelings have been those that formed a basal plate at an early stage. Figs. 46–50 show the early stages of division in a fairly healthy culture of *H. funicularis*. Fig. 51 shows plants from the same culture, but later transferred to fresh sea-water in another dish. The plant in Fig. 52 was grown from the same parent. Within the next month several such plants each produced two upright shoots, and in one case the first shoot bore a lateral branch; rhizoids grew out horizontally from some of the basal plates. These were the strongest *H. funicularis* sporelings obtained, and, like many others, grew on the floor of a dish containing about 150 ml. of sea-water that was not changed for the first 6 weeks. The much-branched and relatively large parent twig, 6 cm. long, was left in the water, where it made a good deal of new growth during this time.

More advanced sporelings were grown only from the plant related to



FIGS. 30–53. Sporeling development. 30–7, *H. congesta.* 30, zoospore (× 1,070); 31, resting spore (× 300); 32, 33, sporelings from a culture 9 days old (× 300); 34–7, from a culture 2 months old (× 150); 34, branched filament; 35, basal plate with horizontal and erect outgrowths; 36, side view of plate and erect growth; 37, large globular mass of spores of which many have germinated. 38–45, *H. brachycarpa* (?); 38–43, from cultures up to 3 weeks old (× 300); 44, 45, sporelings 7 weeks old (× 150); 46–52, *H. funicularis* (× 300); 46, three sporelings, 15 days old; 47, four sporelings, 23 days old; 48, three sporelings, 30 days old; 49, three sporelings, 36 days old; 50, sporeling, 42 days old; 51, three sporelings from same stock, 8 weeks old, grown in more shaded part of same culture for 4 weeks, then transferred to new sea-water; 52, sporeling, 7 weeks old from same stock, but grown along with twig, water changed after 6 weeks. 53, *H.* aff. *platycena*, sporelings from culture 2 months old (× 100), *a* growing epiphytically, *b* and *c* from floor of same glass dish. *ch*, colourless hair; *hp*, horizontal plate; *r*, rhizoid; *sh*, shoot; *spr*, spore; *ss*, probably the newest shoot.

H. platycena which belongs to the section of *Halopteris* with solitary sporangia. Typical examples are illustrated in Fig. 53. There was a striking difference between those attached to the floor of the dish (*b* and *c*) and those in the same vessel which grew epiphytically, either on the parent twig or on a scrap of *Acrosorium*-thallus (*a*). Some of these developed four upright shoots, and one had two laterals on one side. Laterals appeared either on the first-formed or on later shoots.

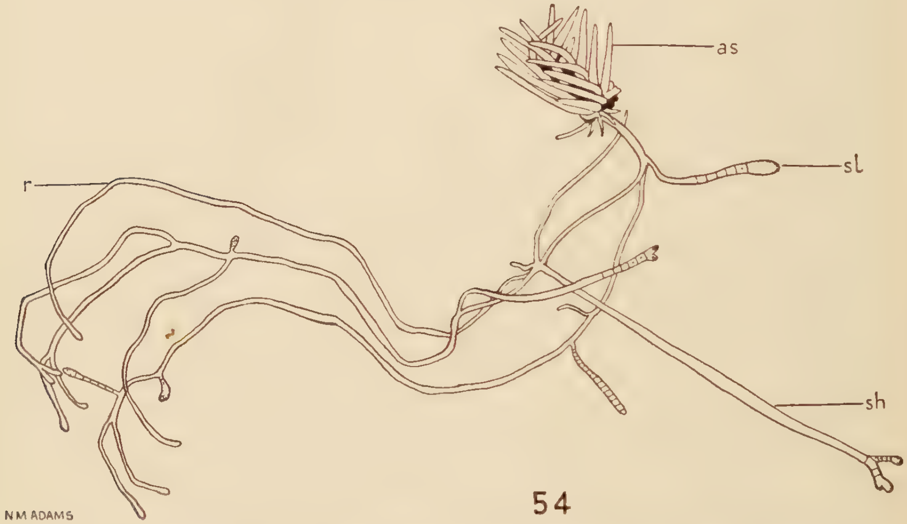


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FIG. 54. *H. spicigera* (× 12). Rhizoids and shoots growing from base of asexual spike dissected from parent plant, 46 days earlier. *as*, asexual spike; *r*, rhizoid; *sh*, shoot; *sl*, apical cell of shoot.

In the best developed sporelings the early stages correspond fairly closely with the figures given by Sauvageau for *Cladostephus verticillatus* (1914, Fig. 122) and for *Halopteris scoparia* (1909). Long colourless simple uniseriate hairs were seen only in *H.* aff. *platycena*, where they appeared rather irregularly, either on the basal disc or laterally or terminally on early shoots (Fig. 53, *ch*).

Later shoots arose, like the first one, from the basal disc. This agrees with Sauvageau's description and figures of *C. verticillatus*, and differs from the same author's account of *H. scoparia*, where the second, third, and fourth shoots arose each as a vigorous lateral from towards the base of the preceding one, as it neared the end of its period of limited growth.

VEGETATIVE MULTIPLICATION

Halopteris plants remain alive in drift for a long time and must often undergo some growth after being torn from their substratum. Any fragments that become anchored in a place suitable for growth for even a few days may by then have produced attaching rhizoids and be capable of developing into independent plants. The plants exhibit striking vitality in cultures, and even those that had travelled for long periods in the post wrapped in paper grew vigorously. Fragments less than 0·5 mm. long grew easily in culture. Almost any cell seems to be able to produce rhizoids and, as in the germling and sporeling, any rhizoid can produce erect shoots with an apical cell, either directly (Fig. 54, *sh*) or after forming a horizontal plate.

DISCUSSION

This study has established that oogamy certainly occurs in some of the larger and more complex southern species of *Halopteris*. Neither sporelings nor germlings were grown to a stage at which production of gametangia or sporangia could be expected; nor has it been possible to make chromosome counts. Nevertheless the facts render it highly probable that a diploid sporophyte bearing unilocular sporangia alternates with a morphologically similar haploid gametophyte bearing antheridia and oogonia.

An account of the taxonomy of the Australasian species of *Halopteris* and of their distribution is to be published elsewhere, but the following facts derived from field studies are of interest in relation to the life-history.

1. Sexual plants have been collected only in localities where closely similar asexual forms abound.
2. Asexual plants greatly predominate in the populations of all species wherever collected.
3. Asexual plants of certain species occur freely in districts where similar sexual plants have never been found.

Asexual plants of *H. spicigera*, the commonest species of *Halopteris* in New Zealand, have been collected in hundreds, but no sexual plants of this species have been found in this country; such plants have been seen only from southern Australia. Sporelings of this species were far less successful in my cultures than those of the other species investigated. Similarly, intensive collecting around Wellington at all seasons has revealed no sexual plants of *H. brachycarpa*, though asexual plants are common enough in drift, both here and elsewhere; in four small collections from Campbell's Bay, Auckland, two sexual plants appeared in one gathering, suggesting that a detailed search in that district might yield more of this gametophyte.

H. funicularis, which is abundant in New Zealand from latitude 38° S. southwards, has not certainly been found with sexual organs, but there are no reliable vegetative characters separating it from *H. congesta*. It is therefore at present uncertain whether *H. funicularis*, like *H. spicigera*, is represented in New Zealand by asexual plants only, or whether its sexual plants are indistinguishable from *H. congesta*.

Whatever the position in *H. funicularis* may be, enough evidence has been assembled to show that, not only are asexual plants in general much more abundant numerically, but also that, in some species at least, they display a much wider geographical distribution. Similarly, only asexual plants of

H. scoparia appear to have been found on British coasts, though there can now be little doubt that Sauvageau did find sexual plants at Saint Jean de Lux in the south-east corner of the Bay of Biscay (cf. Higgins, 1931, p. 352).

The outstanding ability of the plants to multiply vegetatively may permit them to establish and maintain themselves in large numbers far beyond the limits of conditions needed for successful functioning of either asexual or sexual reproduction. The relatively small size and slow growth of the sporeling, compared with the germling, suggest that, where both asexual and sexual plants are present, the asexual ones have a much better start in life and a much greater chance of surviving early dangers.

In one culture large numbers of sporelings, consisting of up to a dozen cells were gathered together in clumps, and destroyed by polystomellids. Newly discharged spores were quite frequently ingested by other protozoa.

ACKNOWLEDGEMENTS

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Observations on the Structure of the Cell Walls of *Valonia ventricosa* and of *Dictyosphaeria favulosa*

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With Plates XIII, XIV

ABSTRACT

The dissection of partially macerated pieces of the cell wall of *Valonia ventricosa* is described. Laminae comprising a very few, or only one, of the component lamellae of the wall have been readily obtained. The occurrence of three directions of fibrillar orientation is confirmed.

In *Dictyosphaeria favulosa* the cell walls show three equally well developed directions of fibrillar orientation, one forming a set of great circle meridians about the cell, so defining two diametrically opposite 'poles', and the others two series of spirals, right- and left-handed, lying obliquely to the meridians at approximately equal angles in the region of 70°–80°.

Single lamellae of *Valonia* walls show only one direction of fibril orientation, which is parallel to the major extinction direction when viewed between crossed Nicol prisms. When stretched transversely to the fibrils these isolated lamellae open out into networks in a characteristic fashion, indicating the presence of some system of lateral linkages between the strands.

The strands of such networks are shown by electron microscopy to be composed of aggregates of microfibrils of indefinite length and of diameter apparently about 100 Å. This is much less than the diameter (300 Å.) of similar fundamental microfibrils previously reported; the difference may arise from the different methods of preparation of the material. Electron-micrographs do not reveal any structural basis for the lateral linkages between fibrils.

The mechanics of the growth of these multi-lamellate walls is briefly considered.

INTRODUCTION

THE cell wall of the green alga *Valonia ventricosa*, presenting as it does a large, easily handled membrane, the product of a single protoplast, largely uncomplicated by the adherence of cell walls produced by adjacent protoplasts, has long been recognized as especially favourable material for the study of the structure of natural cellulosic membranes. Its main features are thus well established: see Preston (1931), Preston and Astbury (1937), Preston, Nicolai, Reed, and Millard (1948), and other work cited by these investigators. In brief, the wall consists of a number of closely adherent but microscopically distinct lamellae, and exhibits a 'crossed-fibrillar' structure. That is, a fragment examined in surface view shows two sets of striations crossing at a somewhat variable angle—usually about 80°—and the application of crystallographic methods of investigation has shown that these two sets of striations

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represent the directions of orientation of fibrillar crystallites of which the wall is built up. In a consideration of the available evidence bearing upon the relationship between the visible fibrillar and lamellate features of the wall Preston and Astbury concluded that the unit lamellae do not individually embody the 'crossed' structure of the complete wall (such as would be the case, for instance, if the two sets of fibrils were interwoven in any way), but each has only one direction of fibrillar orientation. In successive lamellae the orientation alternates between the two directions visible in the complete wall, which consists of about forty such lamellae. The systems of fibrillar orientation have a definite arrangement in relation to the cell as a whole: one of them forms a set of great-circle meridians which define two 'poles', at the base and apex of the cell, and the other a set of spirals lying obliquely to these.

This interpretation of the structure of the cell wall has not hitherto been amplified by the systematic stripping of unit lamellae from the wall for individual study. At the edge of a torn fragment of wall a small area of a single lamella may occasionally be seen, as has been reported by van Itersen (1933), and by Preston and Astbury (1937), but as this is almost invariably disorganized into a mere tuft of unconnected fibrils it gives little information on the structure of the lamella as such. It was therefore of some interest to find, in a stock of long-preserved material of *V. ventricosa*, a state of incipient maceration of the wall, and the opportunity has been taken to investigate this condition. After suitable treatment fragments of the wall have been dissected into laminae comprising a very few or only one of the unit lamellae of which it is composed. The conclusions of Preston and Astbury (1937), based on indirect evidence, have thus been confirmed by direct observation, and some other features of the wall structure have been brought to light.

The cell-wall structure of *Dictyosphaeria favulosa*, another member of the Valoniaceae, has not, so far as I am aware, been described in detail, though its lamellate structure is referred to by Crosby (1903). It is of interest in resembling that of *Valonia*, though certain features are more clearly shown. An account of the plant is given by Børgesen (1913) and is summarized by Fritsch (1935). In the present context it is less familiar than *Valonia*, and a brief description of its structure may thus not be out of place. The plant forms a globose, hollow thallus attached to the substratum by a holdfast; it may reach a diameter of 3–4 cm. This thallus is not a single large coenocyte as in *V. ventricosa*, but is built up of a considerable number of much smaller coenocytes, about 1 mm. in diameter, which in the aggregate characteristically form a single-layered hollow sphere, though in old specimens the original closed spherical structure may become torn open. These component coenocytes of the thallus resemble small *Valonia* cells: they are attached to another by rows of hapteron cells in much the same way as are the coenocytes of *V. utricularis* and in their cell-wall structure the two genera have much in common (see p. 283).

The growth of a *Dictyosphaeria* thallus takes place by characteristic 'segregative' cell-division of its component coenocytes. The protoplast of each of these may round itself off into a number of smaller units, about each of which a new cell wall is secreted. The daughter cells so formed arrange themselves in a single layer within the parent cell wall, grow until they fill the latter, and ultimately rupture it. At the same time they develop hapteron cells which hold them together in close contact so that the general form of the expanding thallus is maintained.

The walls of *Dictyosphaeria* cells are not so readily dissected as are the large expanses of wall furnished by *V. ventricosa*, though their smaller size has some advantage in that the cell wall may the more easily be seen as a whole, and its integral organization is thus more readily apparent.

TECHNIQUE

The material used in this investigation was collected by Dr. F. C. Steward at the Dry Tortugas, and has been preserved, apparently in formalin-sea-water, for upwards of 12 years. Specimens of *V. ventricosa* have been cut into small pieces, washed in very dilute hydrochloric acid to remove calcareous incrustations, and treated in 5 per cent. or 10 per cent. chromic acid solutions at about 40° C. to promote their incipient maceration. After periods varying from a few hours to 2 days the pieces of wall were found to show some obvious partial separation into a small number of thinner laminae, and were ready for dissection. They were washed gently in several changes of distilled water to remove excess chromic acid and then kept until required in 70 per cent. alcohol. Dissection was carried out in water, with the aid of a low-power binocular microscope; laminae removed from the wall were picked up from the water on slides, spread out as flat as possible, and allowed to dry, when they adhered firmly to the slides and could be more permanently mounted if desired. Although the unit lamellae of the wall are but a few tenths of $1\,\mu$ in thickness, they may be readily handled in this way provided they are viewed against a dark background in a very intense lateral illumination.

Such unit lamellae are extremely transparent, are not readily stained, and do not lend themselves to normal microscopic study. As the refractive index of cellulose is close to that of Canada balsam they are almost invisible in this medium, and not greatly more so in other common mountants. They are thus best seen, in the air-dry state, by phase-contrast microscope. A dry mount ensures a maximum difference of refractive index between the cellulose fibrils and the surrounding medium (i.e. air) and so tends to maximum phase-contrast effects: the method, of course, excludes the use of higher power oil-immersion objectives, but was found to be the more effective. Appreciable improvement in the phase-contrast images was obtained by the use of an Ilford 'Micro 3' green filter, which minimizes the residual chromatic aberration of the achromatic objectives used: its restricted pass-band may also be expected to give greater precision to the phase-contrast relationship.

A polarizing microscope has, of course, been used to relate the visible structure of the lamellae to their crystalline organization. Some unit lamellae have also been electron-micrographed (see p. 284).

THE LAMELLAR STRUCTURE OF THE WALL

Dissection and visual examination of the macerated wall in the manner outlined above confirms the deductions of earlier workers. It is clear that a unit lamella does not possess a crossed-fibrillar structure, but shows only one set of striations. Under the polarizing microscope these are seen to lie parallel to the major extinction direction of the lamella: the variable relationships between striation and extinction directions to be seen in multilamellate fragments of the wall, as described by Preston and Astbury (1937), are, of course, here eliminated.

The normal alternation of fibrillar orientation in successive lamellae is also evident; so, too, is the less frequent occurrence of lamellae showing a third fibrillar orientation, a point to which Preston et al. (1948) have drawn attention. This appears as a third set of striations which is much less conspicuous than the two main ones and is seldom clearly seen in mounts of the full thickness of the wall. It is, however, not infrequently visible in laminae comprising approximately 3–6 of the unit lamellae, especially where, as a result of the maceration and dissection processes, some loosening of the structure of the lamellae has occurred (see Pl. XIII, Fig. 3). It seems to be a real feature of the wall, and not merely an artifact resulting from the accidental displacement, during dissection, of a partially detached portion of a lamella belonging to one of the principal striation directions. The lamellae which show it are, however, apparently less frequently produced and are individually thinner than the others. Its orientation in relation to the cell wall as a whole has not been elucidated (but see *Dictyosphaeria*, p. 283).

In the dissection process the wall may usually be relatively easily split into 10–15 laminae, each comprising 2–4 of the unit lamellae, but further dissection of these into single lamellae (each showing one striation direction) is more difficult. It thus seems that the lamellae tend to adhere in groups which are less strongly attached to one another. A similar grouping has been observed in microtomed sections of partially macerated walls, and is also evident in Fig. 8 of Preston and Astbury (1937). Whether this apparent grouping is fortuitous or represents some definite feature of the wall, such as a rhythmic deposition of groups of lamellae during growth, is an open question.

Dissection of the wall also confirms Preston's estimate (i.e. about 40) of the number of lamellae in the wall. It is difficult to obtain an exact figure, even in a small fragment of wall, but counts in the region of 30–40 have been obtained. It appears that the outer layers of the wall may become broken up and more or less sloughed off during growth, a process due perhaps in part to the disruptive influence of the large number of epiphytes commonly present on the outer surface. The exact number of lamellae present in the wall thus probably has no great significance.

It is appropriate here to refer briefly to another manifestation of the anisotropic nature of the *Valonia* wall. Specimens have been noted in which numerous, small, apparently fungal, infections are present. The mycelium consists of very fine hyphae which ramify within the thickness of the wall. Initially their growth has been mainly between the constituent lamellae of the host wall, and at the outer margins of the mycelium, where the latter has as yet had little destructive effect on the organization of the wall, the orientation of the hyphae tends markedly to follow that of the main striation directions (see Pl. XIII, Fig. 4). This recalls the orientation of bacteria growing on the outer surfaces of cotton hairs which has been described by Stanier (1942). In the region of the older part of the mycelium, where it is evident, from the large ragged holes which appear in lamellae stripped from infected walls, that the host-wall structure has been broken down, the selective orientation of the first produced hyphae is obscured by the more random growth of later branches.

In *Dictyosphaeria favulosa* the cell wall has a multi-lamellate structure akin to that of *Valonia*; its most striking feature is the presence of three striation directions which are approximately equally well defined; the previously noted occurrence of a third set of striations in *Valonia* is thus not exceptional. Examination of more or less complete cell walls of *D. favulosa* also reveals the general arrangement of the striation directions and in particular the 'poles' at which the meridional great circle striations converge. In *V. ventricosa* these were found by Preston and Astbury (1937) only after systematic exploration of the wall by X-ray diffraction methods and, as Steward has noted (1939), are not clearly evident from microscopic examination.

In each coenocyte of *Dictyosphaeria* the 'poles' lie in the inner and outer walls (i.e. the walls which are tangential to the thallus as a whole). The outer 'pole' is more easily studied than the inner, since the outer wall is approximately plane, and in it the meridional striations may be seen extending radially from the 'pole', though with some suggestion of curvature, whilst the other two sets of striations appear as right- and left-handed spirals approximately equally inclined to the meridians at an angle in the region of 70–80°.[1] Seen in surface view the 'pole' thus has a structure which is essentially radially symmetrical, and so between crossed Nicol prisms shows a more or less well-defined spherulite cross (Pl. XIII, Fig. 5).

By comparison with this structure revealed in *Dictyosphaeria*, therefore, the third striation direction sometimes visible in preparations of *Valonia* walls most probably has a spiral distribution about the cell as a whole opposite in inclination to the spirally arranged principal striation direction.

THE STRUCTURE OF A UNIT LAMELLA (VALONIA)

A piece of an isolated lamella of the wall, when seen in its normal condition, shows merely one set of parallel striations representing the fibrillar orientation. Such a lamella is very thin by ordinary standards—about $0{\cdot}4\,\mu$,[2] but nevertheless it has a unified structure; clearly, the fibrils which are its conspicuous feature, and which may be seen as isolated strands at a torn edge, normally have some lateral cohesion. It is, of course, very fragile, especially when subjected to tensile stress more or less transverse to the striation direction, and the original parallel orientation of the fibrils may then become disrupted, a process which follows a characteristic course. Long, parallel-sided gaps appear in the lamella, running slightly obliquely (to the right or left) of the striation direction, and these remain traversed in ladder-like fashion by fibrillar strands of variable thickness which, having become partially detached from their neighbours, bridge the gaps at intervals (Pl. XIII, Fig. 1).[1] Under continued tension this process may take place again and again, the 'ladders' appearing between closely adjacent fibrils, so that a network is produced, as in Pl. XIII, Fig. 2. This shows part of a small fragment of a unit lamella, initially composed of parallel fibrils, which has been expanded into a network of many times its original area. It is clear that the network does arise in this way and is not the result of a crossed-fibrillar organization latent within the lamella from which it is derived; its structure is maintained by localized regions of more persistent lateral adhesion between its constituent strands.

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[1] No systematic measurements have been made.

[2] This estimate is based on the assumption that there are 40 lamellae in the wall, which has a total thickness of about $15\,\mu$.

The fibrils of such networks are apparently endless and have no longitudinally recurrent periodic structure. In thickness they exhibit a considerable range, apparently down to (and therefore presumably beyond) the limit of microscopic visibility; they are clearly variable aggregates of much finer sub-microscopic fibrils such as those revealed in the electron micrographs of Preston et al. (1948). These micro-fibrils were found to have a diameter of 300 Å. and might well, by their lateral adhesion into bundles of variable size, produce visible strands such as those of Pl. XIII, Fig. 2, though the electron-micrographs do not suggest such a grouping, nor is any form of lateral linkages between the fibrils evident.

These electron-micrographs were taken from surface replicas of chromium shadowed preparations of the cell wall; the wall itself, or any considerable fraction of it, is far too thick to be electron-micrographed directly. Fibrillar networks, such as that of Pl. XIII, Fig. 2, derived from unit lamellae of the wall, and containing strands of thickness near the limit of microscopic vision, do, however, suggest themselves as suitable material for 'direct' electron-micrography. Such networks have therefore been spread out on the stainless-steel mesh screen object supports used in the electron-microscope, without any additional supporting collodion film, and have been micrographed by Dr. S. G. Tomlin, formerly of King's College, London; examples are shown in Pl. XIV, Figs. 7 and 8. The denser, thicker components of the networks shown here represent on a larger scale the visible strands of photomicrographs (compare Pl. XIII, Fig. 2) and among them appear much finer strands, below the limit of normal microscopic vision, of which, clearly, the thicker ones are multiple aggregates.

The general impression of sub-microscopic structure conveyed by Pl. XIV, Figs. 7 and 8, is at first sight very different from that indicated by the surface replicas of Preston et al., though the differences are resolvable to a large extent. As might be expected, the 'direct' micrographs suggest a much greater compactness of structure than is evident from surface replicas, since the density of the image at any point is a function of the total thickness of material there rather than of its surface relief. Presumably, too, the apparent spaces between the fibrils shown in the surface replicas are occupied in part by inter-fibrillar linkages which, though individually too fine to have a detectable counterpart in the replica, nevertheless collectively contribute to the apparent density of a multiple strand as it appears in a 'direct' micrograph.

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[1] Other fibrous structures, e.g. wood-shavings, may tear in a similar fashion when stressed transversely.

There are, however, certain differences which cannot be disposed of on these lines. Pl. XIV, Figs. 7 and 8 (and other similar micrographs), lack any indication of the linearly arranged spherical structural units described by Preston et al. (1948), and furthermore they do not confirm the constancy of the diameter of the fundamental unit microfibril (*c.* 300 Å.) described by these investigators. Fibrils of diameter down to *c.* 100 Å. appear which suggests that the fundamental unit may be no thicker than this and that the 300 Å. microfibrils are well-defined small multiple aggregates. Alternatively it is possible that the discrepancy in diameter of apparently fundamental units arises from the different methods of preparation of the material for the electron microscope. In the one case the wall has been chromium shadowed prior to the taking of a surface replica, a process which (in view of the thickness of chromium-shadowing commonly used) might tend to exaggerate the apparent diameter of the fibrils at the surface; in the other it has been subjected to chemical treatment and some degree of stretching, which might tend to reduce their diameter. Data relating to other material show similar differences. Thus Hodge and Wardrop (1950) found microfibrils of diameter 50–100 Å. in conifer tracheids, whilst in maize meristems, cotton hairs, and various other fibres Frey-Wyssling et al. (1948) and Mühlethaler (1949) found them to be 250–400 Å. in diameter. All these investigations were based on metal-shadowed material. If there is a microfibrillar unit common to plant cellulose generally, therefore, there is no clear agreement as to its diameter.

The Growth of the Cell Wall

The complex and accurately multilamellate nature of these cell walls presents, besides the problems of their structure, wider questions concerning the dynamics of their growth. The perpetuation of the alternating fibrillar orientation in the many lamellae, throughout increase in thickness and extension in area, is an extremely interesting phenomenon.

The process is evidently different from that which operates, for instance, in the cotton hair, where the deposition of the secondary wall follows, rather than accompanies, the expansion of the hair to its full size. Thus, in *Dictyosphaeria*, I have observed that daughter cells, developing within fully expanded parent cells, and having only $\frac{1}{6}$ to $\frac{1}{8}$ of the volume of the latter, already show in their walls three striation directions, in the characteristic arrangement of the mature cell, though whether the full number of lamellae is present at this stage is not known. In *Valonia*, also, Sisson (1941) has noted that the two sets of striations are present in cells varying in size over a range of about 12 : 1 in diameter; furthermore, the angle between the striations was found to be apparently independent of the cell size. Whilst size differences here cannot, of course, be regarded as necessarily implying corresponding age differences, Steward (1939) considers, from observations of the growth of *Valonia* aplanospores in culture, that the extension of the cell wall in area, and its growth in thickness, must in fact take place simultaneously.

Any considerable extension growth of such multilamellate walls must thus involve the intersusception of new material in the many component lamellae in conformity with the existing pattern, a process difficult to visualize except on the assumption that the growing wall is not sharply differentiated from the protoplast but is laid down within it, i.e. that some component of the latter must permeate the former. This is, of course, a concept of long standing, which has recently been restated, in relation to the primary walls of conifer cambium cells, by Preston and Wardrop (1949). It would seem to be no less applicable to the multilamellate secondary walls of the *Valoniaceae*.

As Preston and Astbury point out, the old idea of a pseudo-crystallization mechanism in the surface deposition of cellulose on the wall is not applicable (at least without serious modification) to *Valonia*. They reject also the hypothesis of van Itersen (1933) based on a rhythmic reorientation of stresses in the expanding cell, and the effect of these on protoplasmic streaming and so on cellulose deposition. The existence of a third direction of fibril orientation clearly introduces a further difficulty here. They visualize, rather, some rhythmic orientating mechanism embodied in the protoplast and cite evidence in favour of this view.

The nature of such a relationship between cytoplasmic organization and the fibrillar structure of the wall is obscure, but a point of some interest in this connexion arises from a comparison with a non-living system. Just as Sponsler (1929) described the growth in thickness of the cell wall in terms of a crystallization process, in which the primary structure of the wall was considered to orientate subsequent surface deposition, so may perhaps the orientation of cellulose units by the protoplast, postulated by Preston and Astbury (1937), be compared with the structural regularity which one crystalline substance may impose on another *included within it*. The selective orientation of crystal inclusions is a well-known phenomenon; an outstanding example of this relationship between a crystalline matrix and inclusions of another substance is furnished by the mineral specimen illustrated in Pl. XIII, Fig. 6. This is a fragment of calcite (a somewhat damaged cleavage rhombohedron) within which, during its crystallization, the deposition of native silver has taken place. The silver takes the form of 'wires' (some of which are multistranded) lying parallel to the cleavage planes of the calcite and forming within it a repetitive, though not entirely regular, 'crossed-fibrillar' system. The arrangement is strikingly reminiscent of that of the *Valonia* wall, though on a much larger scale. The development of this structure thus suggests such a modification of the old idea of the pseudo-crystallization of the wall as may make it applicable to the *Valoniaceae*. The site of the crystallization forces, if they may be so described, may be visualized in the protoplast, rather than in the cell wall, as Preston and Astbury propose—clearly on general grounds a more satisfactory point of view. An examination of the protoplasts of these cells by modern techniques might thus perhaps reveal in them some sub-microscopic organization within which the structure of the cell wall arises and to which the origin of its visible regularity may be attributed.

ACKNOWLEDGEMENTS

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EXPLANATION OF PLATES

Illustrating K. Wilson's article on 'Observations on the Structure of the Cell Walls of *Valonia ventricosa* and of *Dictyosphaeria favulosa*'.

Fig. 1. *Valonia ventricosa.* Single lamella from the cell wall, showing one striation direction, and the characteristic 'laddering' of the fibrils resulting from transverse tension. (Phase contrast, ×450.)

Fig. 2. *Valonia.* Network derived by continued stretching of a single lamella of the wall. (Phase contrast, ×450.)

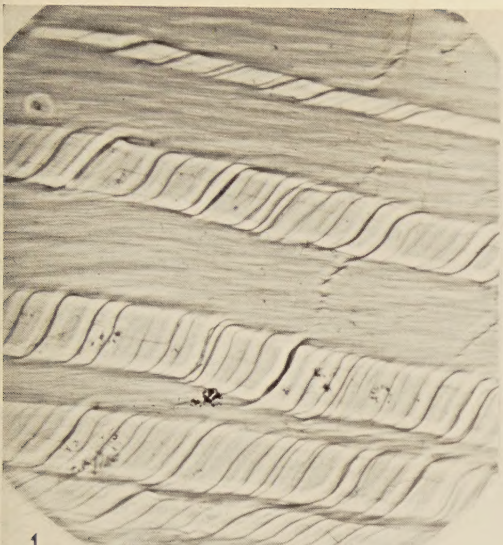
Fig. 3. *Valonia.* Pauci-lamellar lamina from the wall showing three striation directions. (Phase contrast, ×450.)

Fig. 4. *Valonia.* Cell wall with fungal infection, showing selective orientation of the growth of the leading hyphae. (Phase contrast, ×450.)

Fig. 5. *Dictyosphaeria favulosa.* Cell wall of one coenocyte showing three striation directions. The dark cross marks the position of a 'pole' from which one set of striations radiates. (Polarizing microscope, ×280.)

Fig. 6. Cleavage rhombohedron of calcite, with included native silver. The silver forms a 'crossed-fibrillar' system within the calcite, the strands lying parallel to the calcite cleavage planes. Five layers of silver strands, alternating in direction, are represented. (Natural size.)

Figs. 7 and 8. *Valonia.* Direct electron-micrographs of fibrillar networks derived from single lamellae. (×19,000.)



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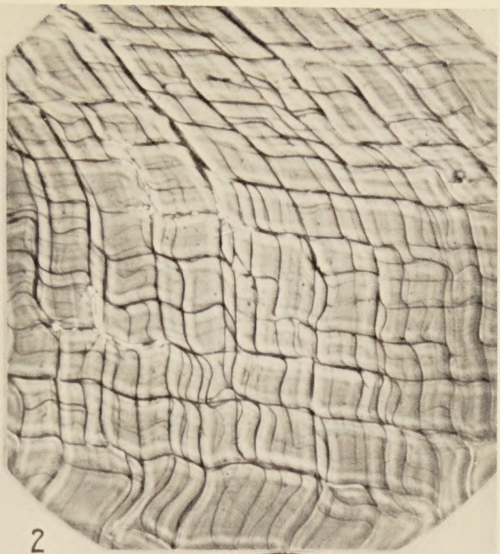

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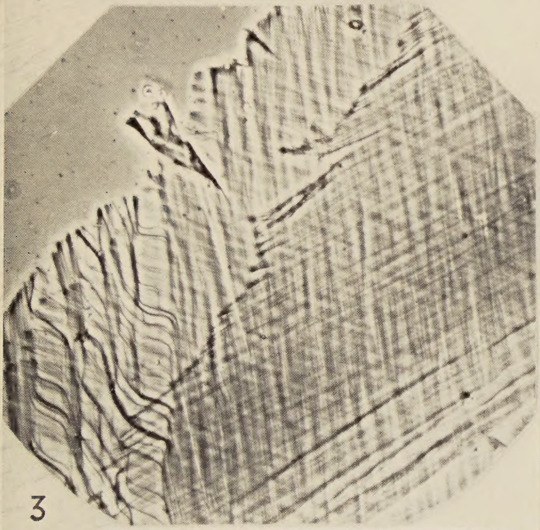

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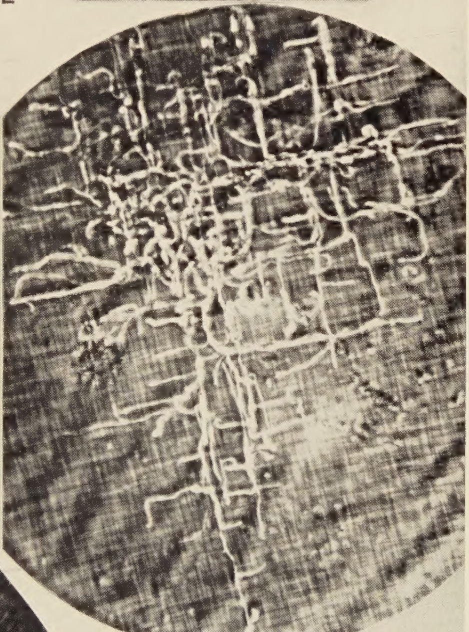

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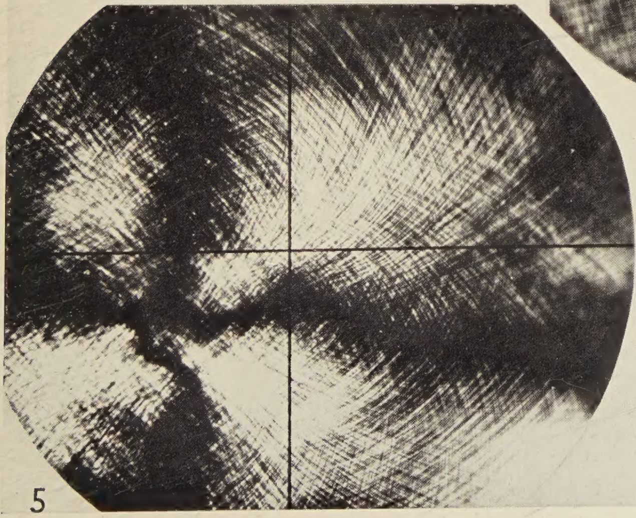

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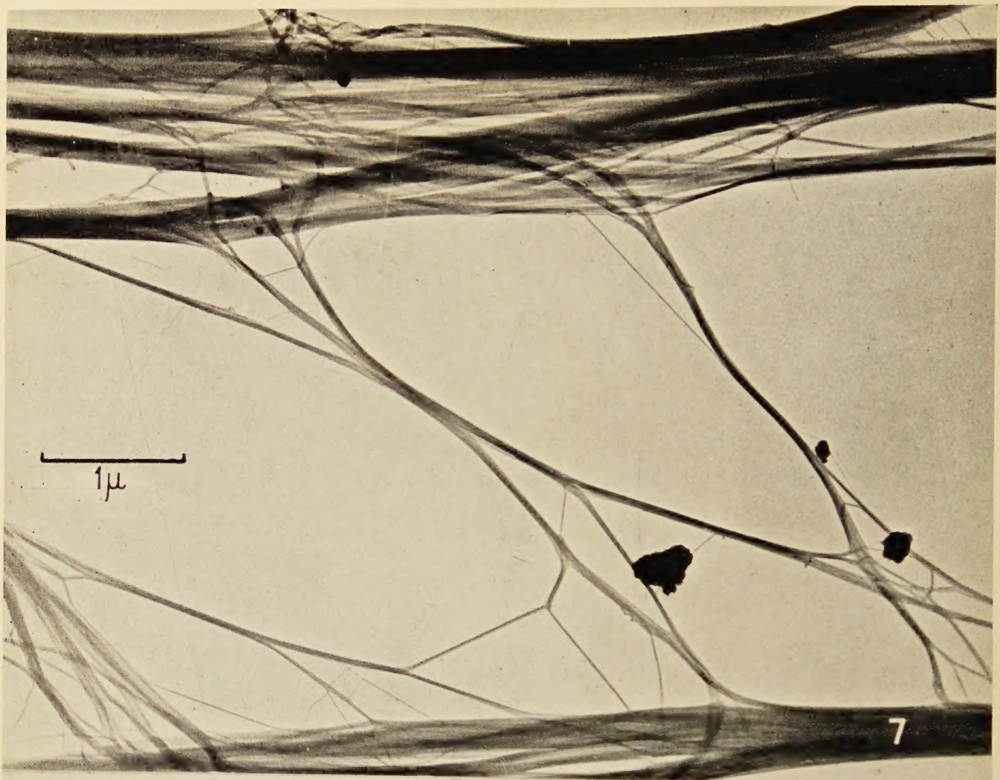

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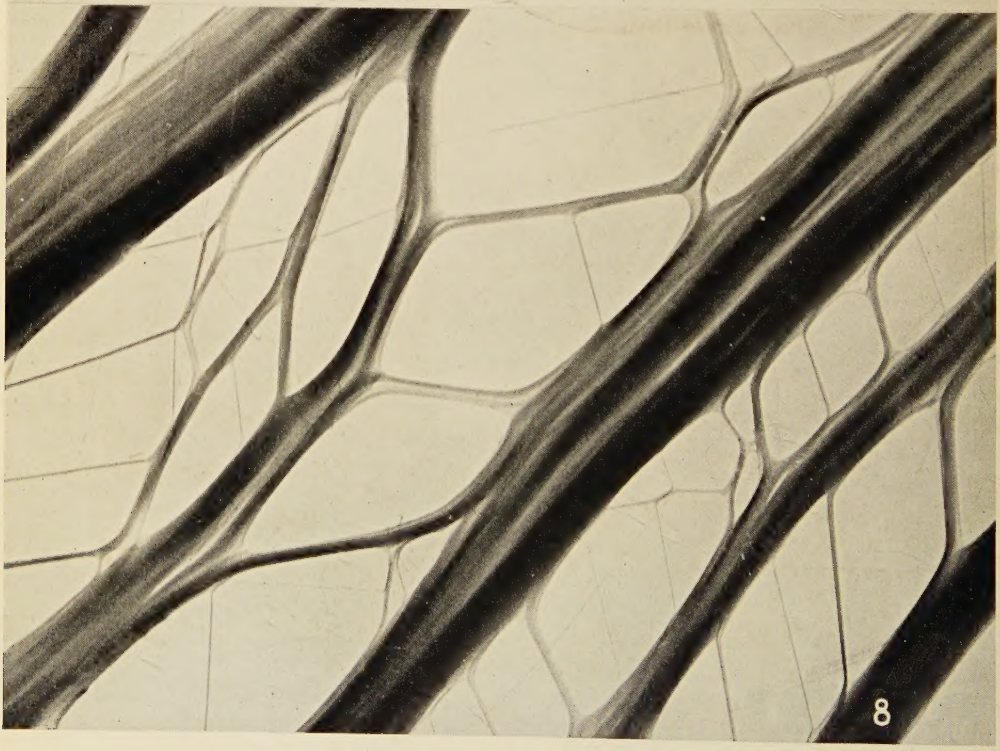

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